Radiometabolic treatment with ^{131}I-metaiobenzylguanidine (^{131}I-MIBG), utilized in conjunction with cisplatin (CDDP) has recently showed promising results in disseminated neuroblastoma (NB) (Eur J Cancer, 31A(4):606, 1995). Possible influence of anticancer agents on MIBG uptake by tumor cells is still unexplored. We measured 125-I-MIBG specific uptake in 4 human NB cell lines SK-N-DZ, SH-SY5Y, KCNR and BE(2)M17 exposed to CDDP, adriamycin or ara-C for 24 and 48 h at concentrations varying from 50 to 400 ng/ml. A significant increase in MIBG uptake after CDDP treatment was observed following 48 h exposure in SK-N-DZ at concentrations varying from 100 to 400 ng/ml ($p<0.0001$), in BE(2)M17 at 400 ng/ml ($p<0.0001$), and KCNR cells at 200 ng/ml ($p<0.05$). In SH-SY5Y cells no difference was observed. CDDP arrested NB cells in G_2/M phase of the cell cycle. A significant increase in MIBG uptake after adriamycin treatment was observed in SK-N-DZ at concentration varying from 50 to 200 ng/ml, in SH-SY5Y from 200 ng/ml ($p<0.05$) to 400 ng/ml ($p<0.01$) and in BE(2)M17 cells from 50 to 100 ng/ml. Adriamycin also induced G_2/M arrest in 3 NB cell lines at concentrations varying from 50 to 400 ng/ml for 48 h. Ara-C exposure did not increase MIBG uptake and as expected a significant block in S phase was noted. Results in vitro demonstrate an increase of MIBG uptake in NB cell exposed to CDDP or adriamycin and suggest a correlation with a specific phase of the cell cycle.

Supported by C.N.R., A.C.R.O., and A.I.R.C.

SL-9

^{131}I-MIBG DENOVO IN PATIENTS WITH NEUROBLASTOMA STAGE III AND IV

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Introduction: ^{131}I-MIBG is effective in neuroblastoma (NBL). It can be used safely in therapeutic amounts in children. The effect is more pronounced in bulky tumours. Thrombopenia is the only serious toxic effect observed so far. These were the most important observations to conclude to the denovo use in neuroblastoma patients where tumour reduction was the first objective.

Protocol outline: ^{131}I-MIBG with a specific activity of 1.48 GBq/mg was administered over 4 hours. This was repeated at least once after 4 weeks. The patients were isolated on a nuclear facility ward. The thyroid was protected by potassium jodide. The parents were mainly involved in the care of their child. This 'pretreatment' was followed by surgery, 4 chemotherapy courses and ablative chemotherapy + ABMT/PBSC in stage IV patients over one year of age.

Patients: 50 patients were included. They all had undoubtfull (pathological confirmed neuroblastoma or ganglioneuroblastoma. They all had inoperable advanced stage disease at any age. Also sufficient uptake in the lesions and no prior therapy except surgery. For diagnosis and follow-up catecholamines, biochemical and biological markers and MIBG scans were available. There were 12 INSS-stage III patients and 38 stage IV patients.

Results: After pretreatment the tumour could be resected for more than 95% (minimal residual disease) in 44% of the patients. In 16% no additional surgery was needed. One patient was lost for follow-up. 34% of the patients are long time survivors. Thrombopenia and especially late recovery after ABMT/PBSC were the main toxic effects.

Conclusions: ^{131}I-MIBG denovo is an effective strategy in advanced stage NBL. This therapy is tolerated well even in patients with a positive bone marrow. During postoperative chemotherapy and ablative therapy recovery of platelets and neutrophils can be delayed. It is difficult to conclude on survival in this relatively small and heterogenous group of patients. Regular consultation of the involved specialists is of the utmost importance.

SL-10

RADIATION ENHANCEMENT BY RADIONUCLIDE THERAPY & HYPERBARIC OXYGENATION.

P.A. Voûte, A. van der Kleij, M.M.C. Tiel-van Buul, J. de Kraker.

A child with a recurrent stage IV neuroblastoma has a bad prognosis over one year. However, the probability to cure a patient with a recurrent neuroblastoma stage IV is considered to be nihil. For these cases one can reach a prolongation of life but it is impossible to reach a cure with chemotherapy. Tumour characteristics, such as hypoxia or metabolic conditions influence the final treatment efficacy.

Because radionated meta-^{131}Iodobenzylguanidine (m-^{131}IBG) has proven to be active in the treatment of neuroblastoma we investigated the application of radiation modifiers combined with m-^{131}IBG to improve the survival rate. Oxygen under hyperbaric conditions was used as a radiation modifier and the determent of the treatment effect was the patients survival treated with m-^{131}IBG alone. At 36 months a survival of 6% was measured for a group of 36 patients who were treated with at least two treatments of m-^{131}IBG. This is compared to a cumulative probability of survival of 40% at 36 months for 41 patients treated with m-^{131}IBG under hyperbaric oxygen. In this group of patients 17 patients are alive.

It may be concluded that unsealed source radiation enhancement by hyperbaric oxygen is clinically feasible. Furthermore, targeted radiotherapy with m-^{131}IBG enhanced by hyperbaric oxygen probably contributes to a better treatment result. These results are promising when compared to the results of the first phase II study on the use of m-^{131}IBG without oxygen.

In the third study on recurrent stage IV patients m-^{131}IBG treatment is done with twice daily treatments for four days with oxygen under hyperbaric conditions.

SL-11

CONCOMITANT USE OF 131-I-MIBG AND CHEMOTHERAPY IN THE TREATMENT OF DISSEMINATED NEUROBLASTOMA.

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Advanced neuroblastoma (NB) has a dismal prognosis. Encouraging results have been reported with 131-I-MIBG used alone in patients resistant to conventional therapy and at diagnosis. We report the first attempt to explore the integration of this new treatment modality with chemotherapy. Cisplatin (CDDP) was initially chosen because it is active in NB and because a synergism between CDDP and radiation has been shown in preclinical studies. Seven patients, 5 with relapsed heavily pre-treated, progressive stage IV NB, and 2 with stage IV NB at diagnosis, all with a good MIBG uptake, were investigated. Treatment consisted of CDDP (50 mg/m^2) i.v. administered in 6 h on day 1 and 8 and 131-I-MIBG (100 mCi) i.v. given in 6 h, on day 2 and 9. Two CR, 2 PR and 1 MR were observed in relapsed patients 4-6 weeks following only one single course of both CDDP and 131-I-MIBG; 1 mixed response and 1 VGPR respectively were observed in the 2 patients at diagnosis. The only toxicity was hematologic, which was significant and relatively long-lasting. In order to reduce toxicity, we have treated 10 additional patients with a modified chemotherapeutic approach, which includes cyclofosphamide (Cy). Both CDDP and Cy were administered a week before 131-I-MIBG, with the aim of reducing radiation toxicity through a "priming" effect. In the 8 relapsed patients, 6 PR, 1 mixed response and 1 MR were obtained. In 2 out of 2 patients treated at diagnosis VGPR and PR were observed respectively. Hematological toxicity was acceptable. The general condition of all patients was excellent. The results of the present study, obtained after a single course of combined therapy, compare very favorably with similar results from the literature obtained with multiple courses of 131-I-MIBG alone over a period of several months. The second

approach should be further investigated, particularly at diagnosis. The final objective of the present study will be the development of a "therapeutic model" at diagnosis in disseminated NB based on intensive concomitant chemo-systemic radiotherapy. *Supported by C.N.R., A.C.R.O., and A.I.R.C.*

SL-12

SUCCESSFUL TREATMENT OF LOCALIZED PRIMARY LYMPHOMA OF BONE IN CHILDREN WITHOUT RADIOTHERAPY.

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Treatment of primary lymphoma of bone traditionally has included irradiation of the primary site (XRT). Recently, we and others have demonstrated that children with early stage non-Hodgkin's lymphoma (NHL) can be treated successfully with chemotherapy regimens of lessened intensity without XRT. We examined results of three consecutive POG studies to determine the impact on outcome of XRT as adjunctive treatment in patients receiving chemotherapy for early stage lymphoma of bone. Among 540 children with early stage NHL entered on POG protocols for early stage NHL between 1983 and 1996, we identified 26 children with NHL confined to a single bone (15 males, 11 females, ages 1-18). Primary sites included femur (9), tibia (7), mandible (4), mastoid (1), rib (1), scapula (1), ulna (1), talus (1), and calcaneous (1). Eighteen children had large cell lymphoma, two had small non-cleaved cell lymphoma, four had lymphoblastic lymphoma, and two had NHL which was not further classifiable. All patients received nine weeks of induction/consolidation chemotherapy including vincristine 1.5 mg/m^2 weekly for seven doses; doxorubicin 40 mg/m^2 on days 1, 22, and 43; cyclophosphamide 750 mg/m^2 on days 1, 22, and 43; and prednisone 40 mg/m^2 daily for 28 days during induction and for five days with consolidation. Seven patients on the first POG study also received 37.5 Gy XRT during induction. These seven patients and four others also received 24 weeks of continuation therapy with daily oral mercaptopurine (50 mg/m^2/day) and weekly oral methotrexate (25 mg/m^2/week), while 15 patients received no continuation therapy or irradiation. One patient with lymphoblastic lymphoma of the ulna treated without irradiation and without continuation treatment relapsed in the testis one year after achieving complete remission. There have been no other treatment failures. With followup through October, 1996, the actuarial event-free survival at 5 years is 95% (SE=6.9%) and at 10 years is 95% (SE=12.0%). No patient has died. We conclude that children with early stage primary lymphoma of bone have an excellent prognosis when treated with chemotherapy regimens of reduced intensity. XRT appears to be unnecessary.

SL-13

LYMPHOMA IN BONE IN CHILDREN

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488 cases of Non-Hodgkins Lymphoma have been treated by the UKCCSG between 1990 and 1996. There have been 12 (2.5%) cases of primary bone involvement and 20 (4%) cases where bone was involved as part of systemic disease. Sites of involvement in the two groups were, in order of frequency for primary tumours spine, knee, pelvis, ribs, femur, upper limb, facial bones and skull, and spine, skull, upper limbs and femur for systemic disease. Presentation in the primary tumours was with swelling, pain, hypercalcaemia, malaise, loss of appetite and cord compression. A single patient sustained a fracture on presentation and there were no fractures later in the course of the disease. Treatment was by systemic chemotherapy in all patients. One patient received radiotherapy (35Gy/20F) initially and one patient on relapse. Of those presenting with bone disease, seven had biopsies, one had a laminectomy, one curettage, one no apparent intervention and one a bone marrow to confirm the diagnosis. No further surgery was performed for any reason. No patients suffered relapse in the primary site and one had relapse elsewhere. Late effects included pain, a limp and amenorrhea. All those presenting with primary disease are alive and well and 85% (17 patients) of those with systemic disease at presentation are alive.

CONCLUSION. Chemotherapy alone appears to be adequate for control of disease in the bone and may avoid some of the sequelae of surgery and radiotherapy.

SL-14

TBI FOR LYMPHOMA

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A variety of different preparative regimens are used in the intensive high dose consolidation treatment of lymphoma. These have been developed empirically and there is no consensus as to an optimal approach. Combinations of drugs with non overlapping toxicities can be used or single or multiple drugs with total body irradiation. Analysis of the clinical outcome of treatment has shown differences in toxicities between regimens, but have not been able to demonstrate overwhelming benefit for one regimen over another.

A rational treatment should be based on established radiological parameters of lymphoma, which at present would be based on data from cell lines. The reported spread of α and β values from these cell lines would give rise to a corresponding spread of log cell kill, which would be produced in different tumours by the same radiation schedule.

Appropriate selection for treatment in individuals would require individual measurement of radiobiological parameters. This is not possible at present and rapid assays need to be developed if this approach is to be useful. Modelling of different strategies with a range of α and β values suggests that mean log cell kill values may not differ greatly between regimens between regimens as diverse as single fraction treatments and fractionated courses giving for example 7 x 2Gy. It may however be possible to select treatment schedules which might be best for a specified range of values.

Other reasons for the failure to observe differences in outcome with different radiation schedules may relate to differences in probability of apoptosis following DNA damage, or varying burdens of tumour cell number initially, of residual disease when clinical complete remission has been obtained.

SL-15

TREATMENT WITH RADIOTHERAPY ALONE IN CLINICAL STAGE I HODGKINS DISEASE

M. Radford and A. Barrett for the UKCCSG

Survival in Hodgkins disease is sufficiently good that the major factor influencing treatment decisions is the incidence of long term toxicity. All chemotherapy regimens used in H.D. have potential toxicity and infertility in males is of particular concern. We have therefore attempted to avoid chemotherapy in Stage I patients.

Method: 370 children aged 3-15 years were entered onto the UKCCSG Hodgkins disease study from 1982 to 1992. 110 patients (88 m. 22 f.) had clinical Stage I disease on the basis of clinical examination, chest x-ray and abdominal C.T. or ultrasound. These patients were treated initially with involved field radiotherapy (35Gy). Patients who subsequently developed progressive disease were treated with ChlVPP chemotherapy (Chlorambucil, Vinblastine, Procarbazine and Prednisolone).

Results:

Alive N.E.D. following R.T. alone	75
Alive N.E.D. following R.T. + chemotherapy	30
Died progressive H.D. following R.T. alone	1
Died 2nd malignancy	1
Died infection during chemotherapy	1
Died unknown cause	1
Died incidental cause	1
	110

Conclusion:
1. 70% of clinical Stage I patients were cured without chemotherapy, preserving normal fertility in males.
2. All patients with progressive disease after radiotherapy responded well to chemotherapy. There is no evidence from these data that disease control is compromised by giving radiotherapy alone to Stage I patients.

SL-16

Bulky Disease and Local Control within Combination Treatment of Hodgkin`s Disease based on the Experience of the German/Austrian Hodgkin Study (HD - 90)

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Purpose: To evaluate the effect of bulky disease on local control within combination treatment of pediatric Hodgkin`s disease (HD) according to the experience of the German / Austrian HD 90 Study.
Patients and Methods: Between 1990 and 1995 563 pts.(315 boys;248 girls; median age12,9years) with HD were treated according to the stage adapted protocol with modified involved field radiotherapy (mIF RT) and chemotherapy. Pts. were treated according to three groups: group I (stage I,IIA) with 2x OPPA(OEPA) and mIF RT 25 Gy; group II (stage IIB,IIIA,IEA,IIEA) with 2x OPPA(OEPA)/2xCOPP and mIF RT 25Gy; group III (stage IIIB, IV, IIEB, IIIE) with 2xOPPA (OEPA)/4xCOPP and mIF RT 20Gy.Pts.with insufficient tumor regression got a local boost of 5 -10 Gy. 495 pts. were eligible for analysis of lymphnode (Ln) size and spread of disease based on clinical and image information (CT, chest X-ray, ultrasound). Bulky disease was defined as lymph nodes > 5cm or a chest tumor > 33% of the thoracic diameter.Tumor relapse was indicated as "infield" or "out of field" or both.
Results: 1062/4950 investigated areas revealed HD with Ln < 5 cm. In 322/1384 involved areas bulky disease was found (52% mediastinal; 39%cervical; 9% axillary, paraaortal, iliacal) with n=92 (28%) in group I, n=95 (29,5%)in group II, n=137(42,5%) in group III. A boost was performed in 84/322 bulky tumors (26%). Relapses were observed in 29 pts.: 17 with bulky, 12 non-bulky HD. The bulky disease group showed altogether 17 pts. with relapses, 4 of them "infield", 4 "outfield", and 8 "infield" and "outfield" (1 unknown). 7/17 pts. with recurrence and bulky disease at diagnosis recurred in the area of the bulk, 1/7 in the bulk area alone, 6/7 additionally in 1-3 areas. The remaining 9/17 did not recur in the area of the bulk, but in other areas (1-2). In one case there was only local relaps of the bulk, in 6 cases a local bulk relaps plus an infiltration of 1 to 3 areas. In the non-bulky disease group, there were altogehter 12 pts. with relapses, 3 of them "infield"-, 4 " outfield relaps" and 5 " infield and out-field" relapses. 1 to 4 areas were involved..One patient had dissemination of hodgkin`s disease.
Conclusion: Bulk seems to have no major effect on local control within systematic combination treatment of pediatric HD according to the experience of (HD 90).

SL-17

CANCER NURSING AND THE ROLE OF RESEARCH

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Research is essential to the development of any profession. Through research, a scientific body of knowledge is generated and theories are developed and tested. The knowledge and theories generated through nursing research are necessary to provide a scientific basis for planning, predicting and controlling the outcomes of nursing practice. The development of a scientific basis for practice through research can lead to professional accountability.
Some people think that research is new to nursing, but Florence Nightingale (1850-1910) initiated nursing research more than 100 years ago in her efforts to improve the care which soldiers received during the Crimean War. Until the 1950s research received minimal attention. Since then, the value placed on nursing research gradually increased. The number and variety of publications, projects, studies and courses have grown at a considerable rate. Community and hospital services have developed research elements as part of overall evaluation, and schools and university programmes include research as part of the cirriculum.
In Europe cancer nursing research practice varies from country to country: in several areas nurses do not have the necessary experience, while in others the number of publications has increased during the last few years. The journals specific to cancer nursing have become sophisticated voices that have attempted to assist nurses as they deal with the difficulties and decisions faced daily in practice. It is important that the cancer nursing research that has been generated make its way into practice. Unfortunately, this is not the case, very little of our current nursing practice is based on research findings. Even today, research findings that clearly warrant utilization are not being used.
This presentation will discuss 1) the historical development of nursing research; 2) the important role that research plays in establishing a scientific base for the practice of cancer nursing; 3) the current status of cancer nursing research; 4) the barriers that preclude research utilisation and 4) possible future directions of cancer nursing research.

GL-1

MOLECULAR EPIDEMIOLOGY OF BURKITT'S LYMPHOMA

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Burkitts' lymphoma differs clinically throughout the world, the characteristic involvement of the jaw in Equatorial Africa being well known. Jaw involvement in the USA and Europe is quite uncommon, but in other world regions, Turkey among them, jaw involvement is intermediate in frequency between these two extremes. We have studied a number of molecular characteristics as well as Epstein Barr virus (EBV) association in Burkittt's lymphoma from various world regions and find significant differences. The pattern of distribution of breakpoints associated with the characteristic 8;14 non-random translocation, for example, is markedly different between Equatorial Africa and the USA, with Latin American countries again showing an intermediate pattern. The factors that determine breakpoint location are unknown, but these differences in different world regions suggest that environmental factors may be involved. Similarly, EBV association is almost invariable in Africa, occurs in less than one third of tumors in the USA and is intermediate, being usually more than 50%, in many other countries.

An important question is why only a tiny fraction of the individuals infected with EBV develop Burkitt's lymphoma. It has been suggested that early infection (i.e., perinatal virus acquisition), which occurs in socioeconomically underprivileged populations, may predispose to EBV associated tumors, but the possibility that specific strains of EBV are associated with tumorigenesis is an alternative explanation. We have recently detected sequence polymorphisms in EBNA1, the only EBV latent gene invariably expressed in Burkitt's lymphoma, that appear to be tumor associated. The most frequently observed polymorphine in peripheral blood lymphocytes (called P-ala, after the amino acid at position 487 in the carboxy region of EBNA1) is rarely oberved in tumors, while a polymorphine not present in peripheral lymphocytes of normal individuals (V-leu) is present in a high fraction of Burkitt's lymphomas. V-leu, however, differs only by one amino acid from a subtype that is sometimes found in normal perhipheral blood lymphocytes (V-pro). Another subtype of EBNA1, P-thr, is found in both peripheral blood lymphocytes and tumors, although the fraction of tumors which carries this subtype varies in different world regions. These findings suggest that P-ala EBNA1 cannot normally contribute to tumorigenesis, and may even be deleterious to tumor formation. The appearance of a new subtype (V-leu) in lymphoid tumors suggests strongly that EBNA-1 is relevant to pathogenesis, a possibility supported by the occurrence of lymphomas in EBNA1 transgenic mice, although the pathways through which it operates remain unknown. One possibility that we are exploring is that polymorphic forms of EBNA-1 have differential protein binding abilities.

These observations, do not support the possibility that there is an oncogenic strain of EBV. Indeed, our data are most consistent with the probability that EBNA1 mutations arise in vivo and are selected for in different cell types. The role of other environmental agents, e.g malaria, remain unknown, but such factors could influence either the likelihood of the development of a chromosomal translocation, or the biology of EBV.

GL-2

GENETICS OF LYMPHOMA

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The lymphomas are the third most common childhood malignancy and account for approximately 10% of cancers in children. About two thirds of the lymphomas diagnosed in children are non-Hodgkin's lymphomas (NHL), and the remainder are Hodgkin's disease. The pediatric NHLs in the Revised European American Lymphoma (REAL) classification include precursor B and precursor T lymphoblastic lymphoma (LL) (30%), Burkitt's lymphoma (BL) (40-50%), diffuse large cell lymphoma (DLCL) (25%). One of the most recurrent mechanism underlying the chromosomal translocations occurring in pediatric NHLs, involves the T-cell receptor (TCR) and Immoglobulin (Ig) loci. Furthermore in most cases partner genes are thought to be transcriptionally deregulated, but leaving the coding region intact. Many of these translocations arise in error during the normal process of the rearrangement of the TCR and Ig genes. In T-cell LL several partner genes have been identified which encode for transcription factor (such as TAL1, HOX11, LIM proteins and others). By contrast in BL the c-myc rearrangement is involved in 100% of the cases. Finally in DCL no major genetic lesions have been so far identified, with the exception of the anaplastic large cell (ALCL) in which the t(2;5)(p23;q35) translocation results in the fusion of the genes producing a chimeric NPM-ALK protein and a minority of B-DLCL showing bcl-6 gene rearrangements. Although the exquisite correlation of certain genetic lesions and phenotype (such as in the BL) may support the pathogenetic role of the involved oncogene, it is likely that multiple genetic lesions (yet to be identified) are necessary to fully recapitulate the complex transformation events. The identification of new genetic lesions and their pathogenetic mechanisms, will be the major goal for future investigations.

GL-3

TRANSCRIPTIONAL REGULATION OF EPSTEIN-BARR VIRUS LATENCY

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Epstein-Barr virus (EBV) infects more than 90% of human adult population in the world. However, it is associated with benign or malignant human diseases including infectious mononucleosis, oral hairy leukoplakia, Burkitt's lymphoma (BL), Hodgkin's lymphoma, T cell lymphomas, cervix cancer, nasopharyngeal carcinoma and gastric adenoma. The dramatic lymphoproliferative capacity of EBV is evident in immunocomprimised hosts (AIDS, XLP, transplant recipients). The role and timing of EBV infection in the pathogenesis of the malignant diseases is not clear. In normal individuals, EBV has developed a delicate balance maintained by oscillating between two latency programs depending on the stage of the host cell and tissue environment. This is accomplished by the choice of distinct promoters that regulate different patterns of viral gene expression under distinct environments. The analysis of the latent gene regulatory DNA sequences and the identification of viral and cellular factors contribute to the understanding of the viral strategy and the timing of EBV infection in the pathogenesis of EBV-associated diseases and helps to develop new approaches to control the viral gene expression.

EBV can establish two different types of latency in B-lymphocytes. In lymphoblastoid cell lines (LCL) derived from normal peripheral blood lymphocytes, six nuclear antigens (EBNA 1 to 6) are transcribed from BCR2 or BWR1 promoters. In the EBV carrying BL cells and in normal resting B-cells only one nuclear antigen, EBNA1 is expressed however from another promoter, Qp.

We have investigated the role of transcription factors in the establishment of distinct latency programs in different B cell phenotypes. The EBNA 1 protein of EBV acts as both a replication and transcription factor through its binding to the two cis-acting elements at the viral origin of latent replication, oriP. These elements are the dyad symmetry element (DS), where replication initiates, and the family of repeats (FR), which is necessary to activate replication at DS and can alone serve as a transcriptional enhancer. The activity of orip FR region directly correlates to the activity of BCR2 promoter. By EMSA, using a tandem repeat region as probe we detected up to 8 complexes in EBV negative and positive cell lines. By cold competition and subsequently by using specific antibodies we identified two of the factors as oct1 and

oct2. In BL cells, only oct1 was bound to oriP and formed a complex with EBNA1. In LCLs however, both oct 1 and oct2 could be detected separately in the complex with OriP and EBNA1. The affinity of oct factors was less than the EBNA1 in competition studies. This is consistent with the noncanonical nature of the oct sites. BL cells contained high amounts of oct1 with none or low levels of oct2. Oct2 is essential for immunoblastic development after antigen triggering and for the activity of B cell specific promoters. The upregulation of oct2 and its involvement in the complex with oriP and EBNA1 correlates with the high activity of oriP, hence the BCR2 promoter utilization in LCLs. It is possible that the prevention of oct2 binding at oriP may be involved in downregulation of oriP activity, hence the downregulation of EBNA2-6 expression in BL cells. The oct binding site at oriP may be "a sensor" for the phenotype of the B cell and the B cell specificity of the infection.

Third EBNA1 binding site lies downstream of Q-promoter utilised in BL cells and resting normal B-lymphocytes. By EMSA analysis using a double stranded probe from this region and an EBNA1 specific antibody we have observed different DNA binding complexes in LCLs, BLs and EBV negative B cell lines. EBV positive or negative BLs showed the same pattern. OriP and EBNA1 competition was observed only with the two complexes that have been observed in LCLs. This data may have implications for the differential regulation of EBV latency in LCL and BLs.

GL-4

EBV and lymphomas

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EBV and lymphomas

Epstein-Barr virus (EBV) is a persistent herpes virus which is carried as a silent infection by the vast majority of the world's population. The virus is associated with certain types of lymphoid malignancies including: Burkitt's lymphoma (BL), nasal T and NK cell lymphomas, B lymphoproliferative disease (BLPD) in transplant recipients, AIDS-associated cerebral lymphoma as well as a subset of Hodgkin's lymphoma, AIDS BL and large cell lymphoma.

In transplant recipients the identified risk factors for BLPD are high dose immunosuppressive therapy and primary EBV infection. The latter is associated with around 50% of BLPD cases, which usually occur in children who were EBV seronegative at the time of transplant and seroconverted while on high dose immunosuppressive therapy. Thus children have a high incidence of BLPD which is fatal in up to 70% of cases.

EBV infection of B lymphocytes *in vitro* leads to their proliferation and immortalisation to yield lymphoblastoid cell lines (LCL). LCLs express 9 latent viral genes of which 5 are essential for the immortalisation process. Since all these gene products are expressed in BLPD cells, it is assumed that this is an EBV driven proliferation. However, since over 90% of the population carry EBV but BLPD is restricted to 1-10% of tumour outgrowth and progression. Data will be presented to show the effects of genetic and epigenetic factors on BLPD outgrowth.

The first line treatment for BLPD is reduction of immunosuppressive therapy which leads to partial or complete remission in most cases. However, recurrences are common, often with progressive resistance to this conservative form of therapy. Thus new treatment strategies are being designed and these include infusion of EBV specific-cytotoxic T cells. The results of a feasibility study in which T cells were grown *in vitro* and infused into solid organ transplant recipients will be presented.

GL-5

NEW ASPECTS IN THE MOLECULAR BIOLOGY OF HODGKIN'S DISEASE

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The mist surrounding the origin and genesis of HRS cells of classical Hodgkin's disease is beginning to dissipate. Molecular biological studies of classical Hodgkin's disease at the single cell level strongly suggest that the HRS cells in the majority of cases represent a monoclonal outgrowth of late germinal centre B cells that have lost their capacity to express Ig. Immunohistological studies showed that in a minority of cases HRS cells express cytotoxic molecules. Those cases might be related to cytotoxic T cells rather than to B cells. Under physiological conditions B cells that are unable to express Ig are eliminated by apoptosis. Most B cell derived HRS cells lost their coding capacity by numerous mutations and should therefore die of apoptosis. Since this is usually not the case, blockage of the apoptotic pathway appears to be a major event in the pathogenesis of B-cell related classical HD. It is tempting to assume that viruses like EBV, as well as genes monitoring the human genome for damaged DNA, like p53, might be involved in the postulated hindrance of the apoptotic pathway, leading to the genesis of classical HRS cells.

GL-6

MOLECULAR ASPECTS OF CHILDHOOD LYMPHOMAS

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Deregulation of cellular growth can be induced on the level of cytokine production, cytokine-receptor expression or post-receptor signaltransduction. Some of these events are induced by chromosomal aberrations.
Recently the t(2;5) translocation was shown to be involved in defined cases of anaplastic large cell lymphoma. The translocation involves a new kinase which is related to the family of insuline or SRC-receptor kinases which are known to regulate cellular growth. Thus it will be of major interest to recognize this translocation in defined lymphoma entities and to study its potential for modulating the cellular growth.
Using RT-PCR we were able to increase the sensitivity in detecting the t(2;5) up to $1:10^6$ cells. Obviously a group of related diseases as anaplastic large cell lymphoma, Hodgkin's disease and lymphomatoid papulosis may carry this translocation. The relation among each other will be discussed.
Furthermore we used substractive cDNA libraries in order to identify new clones which are preferentially expressed in defined lymphoma entities. We isolated ten different cDNA fragments which are highly expressed especially in a celline derived from an anaplastic large cell lymphoma. Among these, there is a new gene which is exclusively expressed in this lymphoma entity but not detected in other neoplasias. The possible relevance to better understand transforming events and to elaborate new diagnostic tools in detecting such exclusive gene expression will be discussed.

GL-7

APOPTOSIS IN LYMPHOMAS

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Apoptosis is the controlled removal of redundant cells during embryonic development, the fine-tuning of the immune system and during organ involution. The cellular suicide programme is under control of genes, many of which being affected in cancer cells. In follicular center lymphoma, translocation (14;18) deregulates the proto-oncogene bcl-2. Increased expression of the bcl-2 gene promotes B-cell survival by inhibiting apoptosis, expanding the population at risk for second mutations leading to malignant lymphoma in animal models. In human lymphoma the picture is far from complete because t(14;18) is not carcinogenic and there is no correlation between the spontaneous occurrence of t(14;18) and the incidence of follicular lymphoma. Overexpression of c-myc, a frequent finding in lymphoma, can induce apoptosis. An important function of Bcl-2 is to antagonise the death signal from c-myc while preserving its growth-promoting properties. Attesting to this concept is the cofactorial role of EBV in the pathogenesis of Burkitts and T lymphoma: transactivation of c-myc expression by EBNA1 with simultaneous suppression of apoptosis by Bcl-2 inducing LMP1 and the Bcl-2 like protein BHRF-1. By contrast, the apoptosis-promoting gene p53, which is dysfunctional in about half of all human tumors, is rarely affected in lymphoma.

Although inhibition of apoptosis predicts rapid expansion of bcl-2 expressing populations, high levels of the anti-apoptotic protein are frequently associated with slowly progressing, indolent disease. This may be ascribed to a simultaneous anti-proliferative effect of Bcl-2 protein, antagonising its anti-apoptotic capacity on the size of the malignant population. A difference in this balance may explain why Bcl-2 levels are correlated with high WBC values in AML but with low WBC in ALL. In leukemia and lymphoma Bcl-2 levels vary widely below and above those in t(14;18)-positive lymphomas. The mechanism involved in the deregulated expression of bcl-2 is unknown but does not involve mutations in the gene. Bcl-2 and related proteins heterodimerise with counteracting Bax and family members. The Bcl-2/Bax ratio would seem, therefore, more critical than the absolute levels of either protein. The clinical value of the Bcl-2/Bax ratio is currently under investigation.

Because overexpressed Bcl-2 protects tissue culture cells against the induction of apoptosis by cytostatic drugs, it has been postulated that the propensity to apoptosis determines the intrinsic sensitivity to cytostatic treatment and that Bcl-2 confers a special type of multi-drug resistance. Studies in vitro have confirmed that the Bcl-2/Bax ratio is indeed critical in the response to cytotoxic insult, notably in case of glucocorticoids in lymphoid and leukemic cells. However, elevated Bcl-2/Bax levels in clinical leukemia and lymphoma are not clearly associated with unresponsiveness to treatment with glucocorticoids. Overall, there are large discrepancies between in vitro studies and clinical observations regarding the role of apoptosis-controlling genes in drug response. An explanation could be that in vitro studies do not account for the effect of these genes on tumorbiological factors, unrelated to apoptosis

In addition to glucocorticoid hormones, ligandation of TCR and CD95 receptors act as specific apoptotic signals in subsets of lymphoma cells. Accordingly, the strategic concept of selective triggering of apoptosis in tumor cells would seem to hold most promise for novel therapies of lymphoma and leukemia.

GL-17

BIOLOGICAL TARGETED THERAPY IN LYMPHOMAS

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B-cell precursor (BCP) leukemia is the most common form of childhood cancer and the second most common form of acute leukemia in adults. Human BCP leukemia was treated in a severe combined immunodeficient mouse model by targeting of the tyrosine kinase inhibitor Genistein (Gen) to the B cell-specific receptor CD19 with the monoclonal antibody B43. The B43-Gen immunoconjugate bound with high affinity to BCP leukemia cells, selectively inhibited CD19-associated tyrosine kinases, and triggered rapid apoptotic cell death. At less than one-tenth the maximum tolerated dose more than 99.999 percent of human BCP leukemia cells were killed, which led to 100 percent long-term event-free survival from an otherwise invariably fatal leukemia. The B43-Gen immunoconjugate might be useful in eliminating leukemia cells in patients who have failed conventional therapy. B43-Genistein was not toxic to monkeys. The Phase I clinical study is now underway in leukemia and lymphoma patients with very promising early results.

GL-8

RADIOISOTOPIC STUDIES FOR DETECTING LYMPHOMAS.

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The contribution of nuclear medicine to pediatric oncology is expanding, e.g. in the fields of tumor imaging, quantitative function analysis of organs at risk during oncological therapy, and radionuclide therapy.

Techniques have changed from analogue spot views to digital imaging of the whole body, showing tumors and metastases regardless of their localisation in a single procedure. In addition, single photon emission tomography (SPECT), 3-dimensional display of images and positron emission tomography (PET) are used.

Radiopharmeuticals have developed from organ orientated, through aspecific tumor-seeking to highly specific tumor-seeking agents, which depict the tumor as a "hot spot", exploiting a variety of metabolic and biological properties of individual tumors. Thereby the focus of interest is shifting from tumor detection to tumor characterisation.

Table 1 shows a list of nuclear medicine applications in lymphoma. Where radiological techniques aim at anatomical delineation of the tumor, nuclear medicine utilizes its functional characteristics.

This survey will focus on radiopharmaceuticals used for imaging lymphoma; the targeting mechanism and the role in the management of the disease will be briefly discussed and illustrated by clinical examples.

TABLE 1	**agent**	**mechanism/role**
organ imaging	Tc99m-phosphonate	bone scintigraphy
	Tc99m-mcolloid Tc99m-NCA-95-MAb	bone marrow scintigraphy
tumor imaging	Ga67-citrate	transferrin
	Tl201-chloride	Na/K-pump,ATP-ase
	Tc99m-sestamibi	mitochondrial upt. p-glycoprotein

	Tc99m-tetrofosmin	idem
	In111-octreotide	SMS receptor
	I123-VIP	VIP receptor
	radiolabeled MoAb	antigen binding
PET/MCD	F18-deoxyglucose	glucose metabolism
	C11-methionine	protein synthesis
organ funct.	Tc99m-HSA/ery's In111-antimyosin I123-MIBG	monitoring cardio-toxicity/cardio-protective agents
	Tc99m-MAA/Kr81m In111-octreotide	monitoring pulm. radiation lesions
	Tc99m-DMSA Tc99m-MAG3	nephrotoxicity radiation lesions
therapy	radiolabeled MoAb	radioimmunotherapy

^{67}Ga-citrate has been used for decades in the detection of lymphoma and has a role in staging, follow-up and early prediction of response. Binding to transferrin and lactoferrin receptors, it also shows inflammation and granuloma.

^{201}Tl-chloride is taken up through the sodium-potassium pump by ATP-ase binding, and therefore represents tumor perfusion and viability.

More recently ^{99m}Tc-sestamibi and -tetrofosmin are used to image lymphoma; these are cationic complexes, which are taken up in mitochondria; they are removed from the cell by p-glycoprotein, associated with multidrug resistance.

Positron emission tomography using ^{18}F-fluoro-deoxyglucose (FDG) and the amino acid ^{11}C-methionin demonstrates the glucose metabolism in viable tumor cells and the tumor's increased protein synthesis, respectively

The peptides ^{111}In-pentetreotide (octreotide) and ^{123}I-vasoactive intestinal peptide (VIP) are used to image neuroendocrine tumors and lymphoma, by binding to the somatostatin receptors or VIP-receptors (89% and 58% of lymphomas, respectively); ^{111}In-pentetreotide is also used to detect radiation damage to the lungs and to monitor the response to corticosteroid therapy.

None of the tracers mentioned above, however, is specific for lymphoma.

A variety of radiolabelled monoclonal antibodies are used for the specific targeting of lymphoma by antigen binding and in adults favorable results have been obtained with radioimmunotherapy using ^{131}I-labeled anti-CD20 antibodies.

Radionuclide tumor imaging may be helpful in the monitoring and early prediction of response, as changes in the tumor's function may preceed anatomical response. Failure to convert from positive to negative is often associated with a poor prognosis.

GL-9

TELOMERASE ACTIVITY IN LYMPHOMAS

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Multiple mutational events that activate oncogenes or delete control genes of genomic aberration are believed to be the main mechanism in carcionogenesis. The evolution of clinically relevant cancers, however, is bound to an uncontrolled growth. This is based on the immortality of tumor cells that perpetuate in mitosis. Telomerase activity has been shown to represent the main mechanism behind indefinite life afflicted to tumor cells. Its measurement provides clues to:

1. definite cancer diagnosis
2. tracing of minimal residual disease
3. perspectives for novel therapeutic strategies

Here we report on our results studying telomerase activity in a large number of cases of malignant Non-Hodkgin-lymphomas, on Hodgkin's diesease and related neoplasms. These findings underline the possibility of early and definite tumor diagnosis, of monitoring minimal residual disease as well as of exclusion of doubtful cases.

GL-10

AIDS RELATED TUMORS

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With the overall goal of greatly expanding our knowledge of pediatric AIDS-related malignancies, several years ago we established a national network of institutions linked to reference laboratories and a statistical/data management center within the Pediatric Oncology Group (POG). Our aims were to foster the systematic investigation of pediatric AIDS-related cancers: their natural history, classification, epidemiology, clinical and laboratory correlates, viral association, and response to protocol-based therapies.

Patients and Methods: We incorporated a case-control design, matching cases of HIV-infected children with cancer to two-control groups: 1) HIV-uninfected children with cancer, and 2) HIV-infected children without malignancy, matched to cases by route/duration of HIV-infection. Clinical histories, as well as tumor and blood specimens, were obtained from all subjects, and investigations included lymphocyte subsets, quantitative viral burden estimation by PCR for HIV, EBV, CMV, HHV-6, and (in some cases) KSHV, as well as pathologic classification, *in situ* hybridization, and molecular characterization of tumor tissues for viral association, immunoglobulin (Ig) gene rearrangements, and mutations and/or translocation of the c-*myc* oncogene.

Results: Of 29 cases of AIDS-malignancies we have studied, the mean age ($\pm$ s.d) was 8.1 $\pm$ 5.7 years, and malignancies observed were: 18 NHL, 2 B(Slg+) ALL, 6 leiomyosarcomas, 2 Hodgkin's' disease, and 1 hepatoblastoma. Cases did not differ from HIV-controls in mode of HIV infection (vertically transmitted vs. acquired), prior anti-retroviral therapy, serologic results for EBV CMV, and HHV-6 antibodies; p24 antigen levels, or CMV or HHV-6 copy number by PCR. Cases had significantly higher EBV viral burdens than controls and significantly lower CD4 cell counts at diagnosis (245 $\pm$ 298/mm^2 versus 451 $\pm$ 476/mm^3 for HIV-controls, $p = 0.02$). As previously reported (*New Engl. J. Med, 332:12-18, 1995*), we detected EBV within clonally-derived smooth muscle tumors from AIDS-related leiomyosarcomas, suggesting a role for EBV in tumorigenesis. Unlike reports from adults with AIDS-associated lymphomas, to date we have observed rather infrequent association with EBV or abnormalities of the c-*myc* oncogene in studies so far completed in 14 pediatric cases of lymphomas and leukemias of B-cell origin. We have also observed 6 pediatric AIDS cases with MALT lesions (Mucosa-Associated Lymphoid Tumors) involving salivary gland, lung/bronchiolar mucosa, and oropharyngeal mucosa, two of which were associated with very high EBV copy number in PBMCs, but not tumor cells, suggesting that EBV may not be directly associated with MALT lesions. MALT lesions appear to expand the spectrum of systemic lymphoproliferative processes in pediatric AIDS patients, from typical pulmonary lymphoid hyperplasia/lymphoid interstitial pneumonitis (PLH/LIP) complex to diffuse large cell NHL. We suggest that MALT lesions should be added to the list of AIDS-indicator pediatric diseases.

Conclusions: Pediatric AIDS-related malignancies differ significantly from those observed in adults, with leiomyosarcomas being the second most prevalent. As new syndromes and malignancies arise among children surviving longer with HIV disease, multidisciplinary collaboration and multi-institutional efforts are needed to better understand the role(s) of host demographic features, infectious agents, various growth factors and oncogenes, the degree of immune suppression, and the risk for cancer. Ultimately better treatment and prevention strategies will be required.

GL-11

USE OF DONOR-DERIVED LEUKOCYTES AND T-CELL LINES CONTAINING EBV-SPECIFIC T-CELLS FOR ADOPTIVE IMMUNOTHERAPY OF EBV-INDUCED LYMPHOMAS COMPLICATING ALLOGENEIC MARROW TRANSPLANTS.

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EBV-induced lymphoproliferative disorders, particularly monoclonal lymphomas, constitute a significant complication of HLA-non-identical related and matched unrelated allogeneic marrow transplants, particularly when administered after selective depletion of T-cells or administration of certain T-cell specific antibodies to insure engraftment or prevent GvHD. Monoclonal EBV + lymphomas are lethal and almost invariably refractory to antivirals and anti-neoplastic agents.

In 1992, we initiated trials exploring the use of unirradiated donor-derived leukocytes for the treatment of these lymphomas. To date, 18/19 patients in our series have been treated with this approach. Each patient presented with a diffuse large cell lymphoma of B-cell phenotype which was of donor origin in each of 10 cases adequately evaluated. EBV DNA was detected by pcr in 19/19 cases tested. Evidence of clonality was demonstrated in 11/13 cases adequate for study by analysis of either rearrangements of immunoglobulin genes or the size of genomic termini of EBV episomal DNA.

The patients were treated with single infusions of PBMC from their normal seropositive marrow donors, providing doses of 2.1-10.0 x 10^5 T-cells, median 5 x 10^5 T-cells. The doses administered were calculated to provide a dose of T-cells 10-fold higher than the dose of 10^5 clonable T-cells/kg that we have found to be the threshold dose for acute GvHD in HLA-matched sibling recipients, but still 10-fold lower than that provided by an unmodified graft. The infusions were well tolerated. Complete clinical and/or pathological resolution of the EBV lymphomas was observed in 16 patients. Clinical remissions were achieved within 14-30 days. Two patients died 8 and 16 days post-infusion from intercurrent idiopathic interstitial pneumonia. At autopsy, there was no microscopic evidence of residual B-cell lymphoma. Instead, previously affected nodes were infiltrated with T-cells of donor type. One additional patient succumbed one week following donor leukocyte infusion for an EBV LPD of multi-organ system failure. Autopsy revealed persistent micro-foci of lymphoma with focal necrosis. Three patients developed acute GvHD (2-Grade I :1-Grade II acute GvHD) which responded to topical or systemic steroids not initiated until at least 4 weeks post-resolution of the lymphomas. Five patients developed limited and 3 patients extensive chronic GvHD, of whom one succumbed late after treatment from sepsis. Three patients relapsed with their own leukemia. No patient experienced a recurrence of the EBV lymphoma. Ten of the 18 survive in sustained remission with no further treatment for 3+ to 42+ months since leukocyte infusion.

We have examined the effects of donor-derived PBMC infusions on the lymphoid populations of the marrow allograft recipients treated, including serial characterization and quantitation of EBV-specific CTLp. Infusion of as few as 1,000 EBV-specific CTL precursors could induce massive expansions of EBV-reactive T-cells in the host with induction of durable and complete regressions of EBV lymphomas, clinically demonstrable as early as 18-21 days following infusion. More recent studies with genetically modified populations of EBV-specific T-cells generated *in vitro* have indicated that up to 30% of the populations of EBV-reactive T-cells generated in the host live within the first 3 weeks post-infusion may be derived from inoculated T-cell populations. Furthermore, these EBV-reactive T-cells may persist in the host for periods as long as 18 months following their infusion. Clinical analysis and studies of *in vitro* expanded populations of EBV-reactive T-cells and their reactivity against EBV-induced human lymphomas xenografted in SCID mice have led to early characterizations of the types of T-cells which can produce the durable regressions observed, their capacity to home to sites of EBV lymphomas and the HLA restricted features of their homing and their tumoricidal activities. Based on these studies, adoptive immunotherapeutic approaches are also being considered for the treatment of other EBV-associated malignancies, particularly nasopharyngeal carcinoma and subsets of patients with Hodgkin's disease, in which tumor cells are demonstrated to express EBV-induced antigens which can be targeted by EBV-specific T-cells.

GL-12

NEW TECHNIQUES IN PATHOLOGY

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The basic principle of pathological investigation of a given tissue specimen is to render a correct diagnosis which enables proper treatment and prognostication of the disease. This is of utmost importance in pediatric oncology, where sophisticated treatment protocols have been tailored for the different types of solid tumors and hematologic malignancies. Depending on the facilities of the institution a number of diagnostic tools is available today to enhance diagnostic and prognostic power.

The cornerstone of every diagnostic procedure is still **conventional light microscopy** after proper procurement of the specimen. This includes the selection of representative portions of the tumor tissue, adequate fixation in suitable fixatives and preparation of different conventional stains. The importance of the extensive application of Giemsa stain, PAS stain, trichrome stain and reticulin stain must be stressed again and again. Many tumors are characterized by their constant PAS positivity, while others are not. Some tumors contain large numbers of reticulin fibrils, while others are devoid of such fibrils. Using these conventional stains on a routine basis most tumors which would not be recognized by H&E stain alone can then be diagnosed with certainty. Whenever possible, tissue should be properly fixed in glutaraldehyde for **electron microscopic investigation**. Although most laboratories rely on **immunhistochemical techniques**, immunohistochemical findings can sometimes be more confusing than enlightning. In these situations a retreat to conventional electron microscopy can often be of great help. The immunohistochemical characteristics of all pediatric tumors and hematological malignancies have been extensively described in the literature. In most cases they have been highly useful in delineating different tumor types and in identifying their histogenetic relationship. Where this information is still lacking **cytogenetics** and **molecular cytogenetics** recently provided new exciting information. Some of this new information may even have relevance for prognostic evaluation. Prognosis in pediatric tumors can be defined by several means including clinical data and data obtained from studies in pathology laboratories. An elegant way of estimating **proliferation** in a tumor specimen is to stain proliferating cells with monoclonal antibodies reacting with all cells in the cell cycle. Additional information can be obtained by submitting these cells for **S-phase analysis** in a flow cytometer. At the same time data are provided on the **ploidy status** of the tumor and on cell morphological parameters including nuclear shape and size. In the context of cell proliferation and cell activity **argyrophilic nucleolar organizer regions (AgNOR's)** have been analyzed in many studies. This morphometric technique enables the quantitation of nuclear chromocenters which represent areas of transcriptional activity.

The most significant achievements among solid tumors have been made for the tumors of the so-called Ewing's sarcoma family, neuroblastoma and rhabdomyosarcoma. Based on the new findings treatment strategies could be further defined.

GL-13

Drug Resistance Mechanisms in Pediatric Brain Tumors and Novel Strategies for Overcoming them.

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Pediatric and adult malignant brain tumors are among the most therapeutically intractable of human neoplasms, and drug resistance, both inherent and acquired, is the most formidable barrier to effective chemotherapy of these tumors. Significant advances have been made in recent years in the clarification of some of the key cellular, molecular and genetic mechanisms that underlie the drug resistance phenotype in human tumors. These studies have shown that the drug resistance phenotype is multifactorial and involves alterations in a variety of genes and their encoded proteins, several of which are also associated with oncogenesis, malignant progression, tumor growth, and cell cycle regulation. The mechanisms that have been shown to be involved in brain tumor resistance include phase II drug detoxification and efflux,

and the repair of lethal DNA crosslinks and their precursor lesions.

The major drug detoxification mechanisms of brain tumor drug resistance are those associated with the glutathione (GSH)/glutathione S-transferase (GST) system. Both GSH and GSTs, particularly GST-pi, are significantly over-expressed in drug-resistant brain tumors. Over-expression of GST-pi has also been associated with increasing histological grade of gliomas. Multivariate analysis, in which age and histology were co-variates have shown high GST-pi to be a strong predictor of poor patient survival. Glutathione conjugation of a number of brain tumor active anticancer agents have been characterized and the GSTs that catalyze the formation of some of the drug-GSH conjugates have been identified. Recent data from our laboratories will be presented on the cloning, characterization and molecular regulation of novel GST-pi gene variants from human malignant glioma cells and on their involvement in both alkylator resistance, malignant progression and determination of glioma patient survival.

Another important mechanism of brain tumor drug resistance involves the ability of the tumor cells to repair the damage induced in their genome by the anticancer agents. The repair of these lesions restores the integrity of the cellular genome and results in survival of the tumor cells. The critical cytotoxic lesions induced by the majority of the alkylating agents active against brain tumors are DNA monoadducts and DNA interstrand crosslinks, and the repair of both types of lesions have been demonstrated in brain tumor cells. The best characterized DNA monoadduct repair mechanism is that mediated by the protein O^6-alkylguanine DNA alkyltransferase or AGT, and involves the removal of O^6-chloroethylguanine DNA lesions induced by 2-cholorethylnitrosoureas (CENUs), such as, carmustine and lomustine. The O^6-chloroethylguanine monoadducts are the precursor lesions to the cytotoxic DNA interstrand crosslinks produced by CENUs. Tumors and cell lines in which AGT is elevated, also referred to as methyl excision repair positive (Mer+), have been shown to be nitrosourea-resistant. Data will be presented on this mechanism of drug resistance in gliomas and medulloblastoma and the use of a specific inhibitor of AGT, O^6-benzylguanine, to sensitize cells of these tumors to CENUs. Finally, the role of cell cycle checkpoint regulation in the repair of drug-induced DNA lesions and in the response of human brain tumor cells to DNA-damaging anticancer agents will be discussed. Results from our laboratory on the structural and functional alterations in two tumor suppressor genes that are involved in cell cycle progression, namely, p53 and p16, and the relationship of these in the perturbation of the cell cycle checkpoints following treatment of glioma cells with BCNU will be presented.

The implications of these recent findings on the mechanisms of brain tumor drug resistance in the development of novel therapeutic approaches, including gene therapy and antisense oligonucleotide therapeutics, for improved therapy of human brain tumors, will be discussed.

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GL-14

BONE MARROW TRANSPLANTS IN ACUTE MYELOID LEUKAEMIA.

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Complete remissions can be achieved in 80% of children with AML. Therapy beyond remission is essential and consists of at least two consolidation treatments using higher dosages than in induction treatments. Strategies for post-consolidation therapy include additional chemotherapy, allogeneic bone marrow transplant (allo-BMT) and autologous progenitor cells (either from BM or peripheral blood) transplant (APCT). Recent studies performed in Europe and U.S.A. using chemotherapy alone show EFS rates of 31% to 49% with DFS post-remission of 40% to 60%.

Although the role of allo-BMT in AML in first remission is still controversial, a series of published studies indicate that EFS rates of 55% to 80% are attained. The pre-transplant myeloablative regimens are diverse, some combining total body irradiation (12 to 14 Gy) with cyclophosphamide or etoposide or melphalan and others combining several drugs:busulfan with melphalan or busulfan *plus* cyclophosphamide with or without the addition of etoposide or other combinations. The relapse rate (RR) is low, between 10% and 20% but the transplant-related-mortality (TRM), due mainly to GVHD, infections or interstitial pneumopathies is still high (10% to 20%). Criticisms are made to the data of transplant centres, mainly selection of patients not including patients relapsing very early, but direct comparison between chemotherapy and allo-BMT made by the Children's Cancer Study Group and the French Society of Paediatric Haematology have shown that differences in DFS rates were statistically significant in favour of the allo-BMT.

As only 25% to 30% of children have a suitable donor, APCT has been used as an alternative to allo-BMT since it permits the administration of myeloablative cytotoxic therapy to all patients and has less toxic effects. This procedure does not have the potential benefit of graft-versus-leukaemia effect and the reinfused product may carry residual leukaemic cells capable of producing a relapse. Results obtained are controversial. On one hand, in some studies of cooperative groups no benefit has been found in terms of survival given that the lower relapse rate was compensated by higher toxic deaths. On the other hand, institutional studies from several countries have shown DFS rates post-transplant similar to those attained with allo-BMT. In our experience with 57 consecutive children diagnosed of AML during the period 1988-95, 34 received an auto-BMT and 19 an allo-BMT after the same induction and two consolidation treatments. The DFS post-transplant was 82% and 84% respectively and survival of all 57 patients was 77%. Analogous results have been found in other similar studies.

Recently, the BFM group has identified two risk groups: good risk (30% of the patients) characterized morphologically and cytogenetically and with good early response to treatment, and high-risk (all the others). In their studies, patients in the good risk group had an EFS of 70% with chemotherapy alone in contrast with 35% EFS for high-risk patients.

In our opinion future strategies will take into account the specific characteristics of the disease (M3,M7 in Down syndrome,M5), cytogenetic findings and other risk factors.

Patients with AML, treated with chemotherapy protocols, who relapse have in BMT, either allogeneic or autologous, the only chance to be rescued. Data from International and European registries show that about 40% of patients transplanted in 2nd remission can achieve a long-term survival. Unrelated BMT should be considered only after early relapses and in some more advanced status. In summary, although indication of BMT in AML in children is considered as an open question, we think that it is well established in high-risk patients (about 70% of all cases) in first remission and in all patients having a relapse in second or further remission.

GL-15

TEN-YEAR EXPERIENCE WITH HIGH-DOSE METHYLPREDNISOLONE AS A DIFFERENTIATION INDUCER IN CHILDHOOD ACUTE MYELOBLASTIC LEUKEMIA

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Although there has been considerable progress in the treatment of childhood acute myeloblastic leukemia (AML) with intensive chemotherapy and bone marrow transplantation the results are far from being satisfactory. Since some myeloid leukemic cells both in human and mice can be induced to differentiate normally, an alternative therapy to induce differentiation of leukemic cells has been suggested in several studies. Retinoic acid (RA), the active metabolite of vitamin A (retinol) as a differentiation agent has been using extensively since the last decade with very promising results in the treatment of patients with APL. RA induces maturation of cells only from patients with APL while other morphologic subtyps of AML patients are unresponsive to RA treatment .However, in various experimental studies glucocorticoid hormones (dexamethasone, prednisolone) have also been shown to induce differentiation of mouse myeloid leukemic cells to macrophages and granulocytes in vitro. In addition, ***differentiation of myeloid leukemic***

cells to mature granulocytes by high-dose methylprednisolone (HDMP, single daily dose of 20-30 mg/kg) treatment with remarkable antileukemic effects have been shown in children with APL and other morphologic subtypes of AML. HDMP combined with other antileukemic agents increased the remission rate and prolonged the duration of remission of children with AML. Recently, we have examined the effectiveness of HDMP combined with low-dose cytosine arabinoside and more intensive chemotherapy in 38 newly diagnosed children with AML [20 of them had extramedullary infiltration (EMI)]. Remission induction therapy consisted of HDMP (20-30 mg/kg, p.o x 14d) + low dose Ara-C (10 mg/m2/d twice, s.c x 14d) +mitoxantrone (MITOX)(10 mg/m2/wk x 4). Then patients received consolidation (VCR+Daun) followed by maintenance therapy for 3 years.

HDMP (30 mg/kg/day, for 5 days) were also given every 3 months during maintenance therapy. Five (3 with EMI) of the 38 patients were early death. Fifteen CR (94%) was achieved in 16 children with no EMI. Fourteen (82%) of 17 children with EMI achieved marrow remission within 2 to 4 weeks with marked clinical and radiological improvements of EMI during this period. Treatment could be stopped in 50% of the children without EMI. Until april 1997, their median duration of remission is 74 months ranging between 70 to 86 months with the exception of one patient who died because of cardiotoxicity. Duration of remission of patients with EMI also prolonged significantly. More recently, ***we have also demonstrated morphologic evidence of apoptosis in children with AML treated with HDMP.*** This could be a possible explanation for the dramatic effect of HDMP on EMI.(orbital, gingival, soft tissue, bone and pleural effusion) of AML patients. In addition, ***short-course (3 to 5 days) of HDMP treatment accelerated leukocyte recovery in chemotherapy induced neutropenic children with AML and ALL*** while they were receiving maintenance therapy, possibly by enhancing the endogenous G-CSF , GM-CSF and increasing the hematopoetic CD34+ progenitor cells in both bone marrow and peripheral blood.

In conclusion, HDMP as a differentiation inducer is a very effective agent in the treatment of patients with AML. The addition of HDMP to conventional antileukemic chemotherapy increased the CR rate and prolonged the duration of remission of children with AML. Further clinical studies are needed to determine whether high-dose glucocorticoids are effective in other malignant diseases.

GL-16

Use of Colony Stimulating Factors in Children: towards a European consensus?

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In 1994 the American Society for Clinical Oncology drew up guidelines for the use of Colony Stimulating Factors (CSF) in adults following a series of expert panel meetings.[1] These guidelines, updated in 1996, recommended that the adult criteria should be adopted for use in children.[2] In 1996/7 a panel of European specialists have developed specific paediatric guidelines based on published literature and the clinical experience of specialists within each of 11 countries. In general, the adult guidelines appear applicable to children, but additional considerations must be taken into account.

Evidence from randomised paediatric trials exists for use as intervention in neutropenic patients with life threatening infections, post autologous bone marrow transplantation, to mobilise autologous peripheral blood stem cells in severe aplastic anaemia together with immunosuppressive therapy for patients with no sibling donor and for severe congenital neutropenia. Less clearly established indications include primary prophylaxis to support dose intensification studies in children with high risk/advanced malignancies; secondary prophylaxis following repeated severe infection +/- infection which is compromising therapy; for slow marrow engraftment after BMT; for drug induced neutropenia in H.I.V. positive patients, and possibly in neonatal sepsis.

Dosage and schedules are adjusted according to the purpose of CSF use. Treatment is generally well tolerated. GCSF appears better tolerated than GMCSF. Possible induction of leukaemia appears to be restricted to long term usage in congenital neutropenias, myelodysplasia and severe aplasia. Where clinical trials have been performed, benefits (reduction of infection, antibiotics and hospitalisation) have outweighed any disadvantages (cost, injections, toxicity).

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P-1

CHROMOSOMAL ABERRATIONS IN RHABDOMYOSARCOMA

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The aim of the study was to investigate the type and frequency of chromosomal aberrations (CA) in rhabdomyosarcoma (RMS) and also to search for possible correlations between the chromosomal pattern and clinical course.

Cell cultures from tumour biopsies and fine needle aspiration (FNA) biopsies were processed for cytogenetic analysis. All cell cultures were harvested within 10 days. The chromosomes were G-banded with Wright stain and karyotypic descriptions were according to ISCN.

Clonal CA were found in 14 of the 18 tumours. An abnormal karyotype was found in 7 of 9 embryonal RMS, in 6 of 8 alveolar and in the sole case of pleomorphic RMS. When the cytogenetic analysis was made on FNA samples the relative frequency of failures were higher.

The conclusion was that CA are detected in all histopathologic subsets of RMS. The biopsies should preferably be obtained from surgical tumour resection and not fine needle aspiration. The characteristic t(2;13) translocation was seen in 2 alveolar RMS but not in any of the other subtypes. In 3 of the embryonal RMS hyperdiploid or hypertetraploid karyotypes with few or no structural rearrangements were seen. In all 3 cases the clinical course was relatively benign, suggesting that certain karyotypic patterns in RMS may be of prognostic significance. Cytogeneteic analysis should be an integral part of the diagnostic examinations of children with RMS.

P-2

IL-2 RECEPTOR LEVEL: A RELIABLE TUMOR MARKER IN PEDIATRIC LYMPHOMAS

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Tumor markers (TM) play a critical role in the diagnosis and response to therapy in several pediatric malignancies. The most common TM used in patients with lymphomas, particularly Hodgkin's Disease (HD), include erythrocyte sedimentation rate (ESR), serum copper level (Cu), and ferritin level (Fe). The soluble form of the interleukin-2 receptor (IL-2R) can be measured using an ELISA and elevated levels have been reported at diagnosis and/or at time of progression in adult T-cell leukemia, hairy cell leukemia, non-Hodgkin's lymphoma (NHL), and HD and in benign clinical conditions such as infectious mononucleosis. In order to determine the utility of IL-2R in monitoring pediatric patients with lymphomas, we examined the levels of IL-2R, ESR, Cu, and Fe in patients with lymphoma diagnosed at our institution from 1993-1996. During this period, 15 patients (12 HD, 3 NHL) had at least one IL-2R level (Dianon Systems, Inc.) performed at the time of diagnosis. In most cases, at least one other tumor marker was measured as well. IL-2R was elevated in 14/15 patients tested (93%), followed by elevated ESR (86%), Cu (55%), and Fe (31%). In those patients who had serial tumor marker levels performed throughout therapy, the IL-2R and Cu levels returned to the normal range in 100% of patients tested. Normalization of the ESR was seen in only 43% of patients tested serially. In the 3 patients who relapsed, the IL-2R level was found to be newly elevated at the time of or prior to relapse. An additional patient with HD was found to have a normal IL-2R level at the time of suspicion of relapse only to have a confirmed relapse 3 months later. IL-2R levels accurately reflected disease status and demonstrated little or no interference from other medical conditions as compared to the ESR. We conclude that IL-2R may be a reliable tumor marker in this group of patients and deserves further prospective analysis in a larger number of pediatric lymphoma patients.

P-3

THE EXPRESSION OF THE CD95(FAS/APO-1) ANTIGEN ON THE CELLS IN CHILDHOOD NON-HODGKIN'S LYMPHOMA (NHL)

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Expression of CD95(Fas/APO-1) antigen (AG), mediating apoptosis, was studied in 1987-1988 using monoclonal antibodies (Mabs) IPO-4 in children with some forms of the pediatric hematological malignanses. At the 5-th International Workshop of Human Leucocyte Differentiation (1995) Mabs IPO-4 were referred to claster CD95. Now after 10 years we can evaluate significance of our investigation. Previously (Med Pediatr Oncol 27:289,1996) reporting about ALL we showed definite prognostic significance of the expression AG.

Patients & Methods: Expression of AG was studied on the neoplastic cells of the 13 patients (pts) with NHL (age 3 - 13 years, 10 male & 3 female). The treatment included chemotherapy ACOP & CNS profilaxis. 9 pts died during 3 - 18 mos after diagnosis and 4 pts are alive(110-120 mos). Lymph node cells (3 pts), blasts from the involved bone marrow (9 pts) and NHL cells from ascitis (1 pt) were investigated prior chemotherapy. Tumor cells were studied apoptic AG as well as Thy-1, CD2, CD3, CD5, CD7, CD10, CD-11b, CD19, CD22 ,HLA-DR, sIg, cIg in immunofluorescence method.

Results: The evidence of AG expression was found in 6 pts out of 13 (46,2%). In 2 immunotype groups the number of pts with expression of AG was noted: B-NHL - 4/7 pts and T-NHL - 2/6 pts. At present time 1 out of 7 B-NHL pts and 3 out of 6 T-NHL pts were alive We have noticed significantly ($p<0.01$) higher frequency of AG expression in the group with the treatment failure (6 out of 9 pts) vs. survived group (0 out of 4 pts). We did not found correlation between apoptic AG and others.

Conclusion: The results conform our previous data on prognostic role of CD95(Fas/APO-1) antigen (unfavourable factor on childhood NHL) and need further consideration.

P-4

CYTOGENETIC STUDY USING FISH OF A DIFFUSE LARGE CELL LYMPHOMA IN A CHILD.

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We report the cytogenetic and pathological study of a high-grade lymphoma in a 13-year-old boy.

The child presented with a large inguinal mass. Ultrasound showed numerous inguinal and iliac adenopathies and a nodular splenomegaly. Bone marrow and cerebrospinal fluid were clear of malignant cells. The biopsy of the inguinal lymphnode permitted pathological and cytogenetic analysis.

The pathological findings were "tumoral proliferation of lymphoid cells

with sheets of undifferenciated large cells". Some cells were centroblastic-like partially cleaved. The cells were CD20+ and negative for T-cell markers (CD3-,CD45RO-).
The cytogenetic analysis revealed a modal number of 49 chromosomes with structural and numerical clonal abnormalities. Fluorescent in situ hybridization (FISH) using composite probes of chromosomes 1,6,7,8,11,12,13,14 and 18 and centromeric probes of chromosomes 14/22 and X precised the abnormalities : disomy X, partial deletion of the long arm of chromosome 6 (6q-), trisomy 12 and additional material on the long arm of chromosome 14 (14q+) which did not derived from a t(14;18) translocation.
After two-months of chemotherapy according to LMB 96 protocol, a second look surgery did not find any residual tumor. Histopathologically, there were large necrotic areas in spleen, iliac and femoral nodes without any viable tumoral cell.
FISH is a usefull technique to complete the cytogenetic analysis. Diagnosis and prognosis factors can be brought out. In this case, the ploidy of 49 chromosomes and the 6q- did correlate with diffuse large cell non-Hodgkin lymphomas and the association of 14q+ and trisomy 12 suggested an aggressive tumor.

P-5

CELLULAR AND VIRAL ONCOGENE EXPRESSION IN TUMOR CELLS OF ARGENTINE PEDIATRIC HODGKIN'S DISEASE

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A new category of oncogenes regulating apoptosis, p53 and *bcl-2*, and the Epstein Barr virus (EBV) latent membrane protein-1 (LMP-1) have been related to Hodgkin's disease (HD) pathogenesis. We attempted to determine p53, mdm2, p21/WAF-1, bcl-2 and LMP-1 immunohistochemistry expression in tissue sections from formalin-fixed, paraffin-embedded lymph node biopsies of pediatrtic HD. P53 was detected in the nucleus of Reed Sternberg cells and their variants (H-RS) in 68% of the HD cases. But, there was no statistically significant association either with clinical stages or with histological subtypes. P21/WAF-1, an indirect marker of p53 functional status, showed nuclear labeling of H-RS in all the studied cases. MDM2 co-expressed with P53 in 62% of the cases; this suggests that both proteins are regulated each other, in HD, by a self regulatory loop. *Bcl-2* cytoplasmatic expression in H-RS was demonstrated in 65% of the cases. There was co-expression of *bcl-2* and p53 in 51% of them, but it did not correlate with a poor prognosis. LMP-1 labeling was shown in 51% of the cases, having a statistically significant association with the group ≤ 6 years old (p=0.005). Since LMP-1 induces the expression of *bcl-2 in vitro*, the relation of both proteins was analyzed; exhibiting co-expression in 15/37 cases, with a statistically significant association only in the group of patients ≤ 6 years old. The abnormal accumulation of these oncoproteins in tumour cells can exert a significant role in the pathogenesis of a subset of pediatric HD.

P-6

DETECTION OF CYTOGENETIC ABNORMALITIES IN CHILDHOOD MEDULLOBLASTOMA BY COMPARATIVE GENOMIC HYBRIDIZATION

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Objective:Identify genetic alterations in a series of medulloblastomas in children and see whether these alterations, if present, are of prognostic value.
Methods: Medulloblastomas of children were analysed by comparative genomic hybridization (CGH) to look for genetic alterations. The results of the CGH are being verified by Southern Blotting. Survival and event-free survival of patients will be correlated with the genetic alterations observed.
Results: All tumors (10) showed chromosomal aberrations. Chromosome 17 was most frequently affected. Alterations consistent with Iso (17q), amplifications and deletions were observed in 8/10 tumors.
Chromosome 1p was also frequently lost (4/10). Other chromosomes involved are 9 and 13, for both amplifications and deletions were observed. Chromosome 10 was lost in 3/10 and 2 tumors showed and amplification of 2p in the region were N-myc is located.
Southern blot is being performed now to verify these alterations and interesting regions will be further analysed.

P-7

t(3;17)(q21;q25) IN EPSTEIN-BARR VIRUS ASSOCIATED PERIPHERAL T-CELL LYMPHOMA

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Epstein-Barr virus (EBV) has been associated with lymphoproliferative diseases in immunocompromised or immunocompetent patients. More recently, EBV has been implicated in the pathogenesis of T-cell lymphoproliferations such as peripheral T-cell lymphomas (PTCL). However, EBV-associated PTCL have rarely been reported in childhood and chromosome abnormalities have not been described in such patients. We report on an immunocompetent 8-year-old boy with serologic profile indicating chronic infection by Epstein-Barr virus (EBV) who developed subcutaneous, digestive and pulmonary lesions related to a peripheral T-cell proliferation. No clonal rearrangement of T-cell receptor or immunoglobulin genes were seen. However, the finding of a t(3;17)(q21;q25) in 44 metaphases from one skin lesion demonstrated a clonal origin. Using in situ hybridization technique, we also showed that the proliferative T-cells contained EBV genome leading to the diagnosis of EBV-associated peripheral T-cell lymphoma.
To our knowledge, the presence of a t(3;17)(q21,q25) was not previously reported in such an hematologic disease. Abnormalities of chromosome 3 such as inversions or translocations involving the long arm at band 3q21 are associated with hematologic disorders of the myeloid lineage. This provides evidence that alteration of chromosome 3q21 can be critical for the development of malignancy. Further cytogenetic and molecular analysis are needed in the search for the location of a putative gene related to EBV-associated peripheral T-cell lymphoma.

P-8

P53 AND BCL-2 PROTEIN EXPRESSION IN CHILDHOOD BURKITT AND BURKITT-LIKE LYMPHOMAS: CORRELATION WITH SURVIVAL TIME

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Objective: Apoptosis related genes such as p53 and bcl-2 have received increasing attention in carcinogenesis, drug and radiation sensitivity and

patient survival. We planned to determine p53 and bcl-2 protein expression and their impacts on survival time in childhood Burkitt (BL) and Burkitt-like (BLL) lymphomas.
Methods: Fifty paraffin-embedded specimens obtained from the patients with abdominal lymphoma were investigated by immunoperoxidase method for detection of p53 and bcl-2 protein alterations using the monoclonal antibody DO-7 and Clone 124 respectively.
Results: The overall p53 and bcl-2 overexpression were 48% and 40% respectively. There was no significance between overexpression of p53 and bcl-2 and response to induction chemotherapy, stages in the studied children, majority of whom were in stage III. bcl-2 expression was significantly higher in BLL group than BL. Although we could not show any significant difference, there was a tendency that patients who were either p53 or bcl-2 positive had higher survival rate. Additionally, relapse rate was significantly higher in p53 negative group compared with p53 positive group.
Conclusions: These results suggest that the children diagnosed high grade lymphoma with p53 and/or bcl-2 protein alterations may have a better response to overall chemotherapy, possibly because the lymphoma cells can not arrest in G_1 to correct lethal damage induced by chemotherapy. That Epstein-Barr virus prevalence is high in Turkish children may be one of the factors playing role in high protein expression of p53 and bcl-2.

P-9

QUANTITATIVE DETECTION OF MINIMAL RESIDUAL DISEASE IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA BY INVITRO AMPLIFICATION OF REARRANGED T-CELL RECEPTOR δ CHAIN SEQUENCES

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We studied six children with T-cell ALL. T-cell ALL was classified on the basis of their lymphoid morphologic appearance and the immunophenotype as assessed by flowcytometry. ALL was defined belonging to the T lineage based on the presence of at least two of the CD2, CD5 and CD7 antigens. For the amplification of TCR Vδ1Jδ1 rearrangements a two step semi-nested PCR approach was applied. This procedure allows the detection of a single template molecule of about 400 bp after gel electrophoresis and ethidium bromid staining. The bands were excised from preparative 2% agarose gels, purified and then completely sequenced. The complete Vδ1Jδ1rearrangements contained large junctional regions with an average size of 32 nucleotides and 8 deleted nucleotides. According to the junctional diversity patient-specific primers were figured out for all six patients. Using limiting dilution and replicate reactions at each dilution, we optimized to generate all six patients' results. PCR amplification of DNA from diagnostic leukemia cells from six patients yielded at least one band corresponding from Vδ1Jδ1 rearrangement. Using this limited dilution technique and patient-specific primers PCR was applied to bone marrow samples for all six patients every three months. At the end of 18 months of therapy, two of these patients (Pts 1,4) had detectable residual disease at the level of $1.7x10^{-4}$, even though they are in clinical complete remission. We suggest that MRD negativity at the end of therapy might be an important factor for long-term disease-free survival, because the negative cases had a very low risk of relapse. Because the outcome for MRD-positive cases is more difficult to evaluate, patients with MRD after termination of therapy should be monitored.

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P-10

AN ANTI-N-MYC PEPTIDE (GVAPPRPGGRQTSGGDH) ANTIBODY (IgG) REACTS WITH WATER-SOLUBLE RECOMBINANT N-MYC ONCOPROTEIN

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The importance of determining N-Myc protein rather than genomic N-myc amplification has been emphasized in neuroblastoma. In order to develop an ELISA for N-Myc protein quantification, an effort was made to raise antibodies specific for N-Myc, and also to produce a relatively smaller-sized N-Myc protein, which should be water soluble.

N-Myc-specific peptides, HGRGPPTAGSTAQSPG (codon.136-151) and GVAPPRPGGRQTSGGDH (codon.223-239), were synthesized and injected into rabbits in conjugation with either hemocyanin or lysine core (multiple antigen peptide method). Synthesized peptides conjugated to the lysine core raised more potent antibodies. IgG purified on an affinity column on such peptides showed a precipitation line identical to that of N-myc protein by immunoblot analysis of several neuroblastoma cell lines positive for N-myc. The purified IgG against GVAPPRPGGRQTSGGDH also strongly stained the nuclei of various neuroblastoma tissues and cell lines with N-myc amplification; thus a polyclonal antibody specific for a synthetic peptide from the N-Myc protein was obtained.

For preparing standard protein, partial exon 2 and exon 3 of the N-myc was cloned and inserted into an expression vector, pET16b. A water-soluble recombinant N-Myc protein with a molecular weight of 38 kDa was expressed by the E. coli, and was purified with Ni^{2+} affinity column chromatography. Immunoblot analysis showed the purified anti-GVAPPRPGGRQTSGGDH IgG reacts with the rN-Myc protein. Thus, a water-soluble rN-Myc was successfully obtained.

These results are to enable the establishment of a quantitative measurement of N-Myc protein for the first time.

P-11

THE VALUE OF SERUM CYTOKINES IN CHILDHOOD M. LYMPHOMAS

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During the last decade, consistent data have been produced demonstrating the existence of functional relationships between immunity, inflammation and haematopoiesis. As a result the list of polypeptides involved in the regulation of these systems has grown rapidly.To clarify the role of cytokines in the pathogenesis and the prognosis of malignant lymphomas, three cytokine activities, interleukin-2, (IL-2), soluble interleukin-2 receptor (sIL-2R), and tumor necrosis factor-alpha TNF-α and ferritin were analysed by means of sandwich ELISA technique in 74 children with newly diganosed malignant lymphoma (50 patients with Hodgkin's disease, 24 with non-Hodgkin's lymphoma). Previously recognized factors such as histology, clinical stage, ESR, LDH, trace element measurements were compared with serum cytokine and ferritin levels. The study group consisted of 20 girls and 54 boys with an age range of 3.5 - 18 years (median 8 years). ELISA assays for sIL-2R , TNF-α and ferritin yielded a markedly elevated levels ($p<0.001$) in patients with active disease as compared to remission and control groups. Among these sIL-2R and TNF-α appear to be reliable markers to reflect the tumor burden, thus the highest levels have been correlated with the most advanced disease stage. The role of those circulating cytokines in patients with malignant lymphoma is unknown.Since the sIL-2R is capable of binding IL-2,it may compete with its counterpart on the surface of lymphocytes for the ligand , thus down-regulating the host's anti-tumor immunity. As far as the elevated serum TNF-α levels are concerned, since the patients with infectious diseases have been excluded from this study, these high levels indicate that TNF-α was produced in lymphoma patients either by the malignant cells themselves or the reactive bystander cells. The strong association of TNF-α levels with clinical and biological parameters reflecting tumor burden suggests that tumor cells rather than the reactive cells may be the major source of TNF-α . Indeed, it has been shown previously that TNF-α messenger RNA can be found in lymph node samples of patients with various lymphoid malignancies.
Since there is the availability of recombinant cytokines for clinical use in haematologic and lymphoproliferative malignancies, it is crucial to understand their spectrum of interaction in order to select the appropriate combination for in vivo administration.

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P-12

MICROSATELLITE INSTABILITY AND P53 MUTATIONS IN SECONDARY PEDIATRIC NEOPLASMS

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Nine children with secondary malignancies were studied. The primary tumors were retinoblastoma, neuroblastoma, lymphoma, craniopharyngioma, Wilms' and carcinoma . The secondary tumors were osteosarcoma, leukemia, lymphoma, and glioblastoma. We examined extracted DNA from the primary and secondary tumors for genetic alteration at the p53 gene as well as 7 separate microsatellites localized to the following chromosomes: 17p (p53 gene a dinucleotide repeat), 14p (D14S426 a dinucleotide repeat) retrieved from the GDB data base; 13p (Rb gene a dinucleotide repeat), 15q (GABRB3 a dinucleotide repeat) and 19p (DMI gene a trinucleotide repeat). BAT 25 and BAT 40 are randomly chosen microsatellite markers consisting of an (A)n repeat. We found p53 mutations in 8 patients and microsatellites instability was identified in 5 to 7 loci in the secondary tumors of all patients. Though small number, our findings differ from two recently published data that showed no instability or a very rare microsatellite instability (one out of 54 samples) in primary pediatric tumors. We believe that this is explained by the fact that our patients were selected by the development of an additional tumor, an extremely rare event. This suggests that these children have a mutator phenotype, predisposing them to the development of multiple tumors.

P-13

A COMPARISON OF THE EFFECTS OF DEXAMETHASONE AND CISPLATIN ON CHONDROCYTE PROLIFERATION *IN VITRO*.

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OBJECTIVE: Clinical data collected over several years suggest that cytotoxic chemotherapy in the treatment of childhood malignancies has deleterious effects on the growth plate resulting in short stature. The present study focuses on the effects of cisplatin and dexamethasone, both commonly used in the treatment of childhood diseases, on proliferation and/or differentiation of rat tibia epiphyseal chondrocytes *in vitro*.

METHODS: Measurements were made using a combination of flow cytometry, a 96 well plate colorimetric assay of cell proliferation and colony forming efficiency (CFE) in suspension culture

SUMMARY: In monolayer cultures, cisplatin caused a dose-dependent reduction of chondrocyte proliferation, which was the result of a G2/M cell cycle arrest and subsequent cell loss. Cell loss was >70% at 1µg/ml cisplatin, with little recovery of proliferative capacity of the remaining cells. In suspension culture, cisplatin (1µg/ml) completely abolished the CFE of the chondrocytes. Dexamethasone, however, decreased chondrocytic proliferation only when a threshold concentration of 0.01µg/ml was reached, after which there was no further dose dependency. This was not due to cell cycle arrest or cell loss although the doubling time of the cells was increased at least 10 fold. Compared with controls, 0.1µg/ml dexamethasone reduced CFE by ~95% although single cells remained in culture, with features of chondrocyte stem cells. Once dexamethasone was removed from these cells, they increased their rate of proliferation with time in culture and responded to the stimulatory effects of both Growth Hormone and Insulin-like Growth Factor 1.

CONCLUSIONS: Cisplatin is cytotoxic to cycling chondrocyte cells *in vitro* whilst dexamethasone has a cytostatic effect on these cells, which may be dependent on their differentiation status. This may be significant when estimating the contribution of treatment schedules, involving both cytotoxic and cytostatic agents, to the subsequent recovery of growth plate chondrocytes and hence the rate and extent of longitudinal bone growth *in vivo*.

P-14

FLOW CYTOMETRIC ANALYSIS OF LYMPHOCYTE MARKERS AND CELL PROLIFERATION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA OFF-THERAPY.

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Treatment of childhood Acute Lymphoblastic Leukemia (ALL) provides several protocols of chemotherapy that cause suppression of immune cellular response, with quantitative modifications of some immunological parameters and in vitro qualitative alterations of lymphocyte function .
The mechanisms are not yet clear. This study was therefore deviced to evaluate some functional aspects of immune cellular response of children affected by ALL in continuous complete remission after suspension of the therapy.
A group of 15 patients affected by ALL (14 B-ALL, 1 T-ALL) was studied. Mean age at diagnosis was 50.3± 24 months. They were treated according to AIEOP protocol 9102 and were off therapy for 21.8±9.4 months. Mononuclear cells were isolated from heparinized blood and stimulated in culture with mitogens (CON-A, PWM). To evaluate cellular proliferation measured by DNA synthesis, BrdU was added 18 hours before cellular harvesting. Mononuclear cells, before and after stimulation, were labelled with conjugated monoclonal antibodies and analyzed with flow cytometry (FACScan, B.D.). Antibodies used were CD2, CD3, CD4, CD8, CD19, CD16/CD56, CD25, CD69, CD71,HLA-DR. Lymphocyte activation was evaluated by means of markers co-expression as CD3/DR, CD25/CD4/CD3,CD4/CD69/CD3,CD8/CD69/CD3,CD19/CD69,CD19/CD25.
BrdU incorporation was estimated with a specific monoclonal antibody and in association with propidium iodine (PI) staining in biparametrical analysis.
We have observed a reduction of absolute number of T lymphocytes (CD3+, CD4+) and increasing levels of activated lymphocytes (CD3+/HLA-DR+). After PWM stimulation, there was a marked reduction of expression of activation molecules as CD69, CD25, HLA-DR. There was no evidence of similar results after CON-A stimulation. Data about BrdU incorporation and PI staining clearly demonstrate a low cellular proliferation of PWM stimulated lymphocytes. All data were statistically significant versus healthy controls.
Our results suggest a long lasting depression of immune function after chemotherapy. This cellular defect might depend on an altered T-helper subset differentiation (Th2 ?) that is a critical point for the production of several cytokines regulating the immune response.

P-15

THE USE OF A MAJOR HISTOCOMPATIBILITY COMPLEX NONRESTRICTED CYTOTOXIC NK CELL CLONE FOR THE PURGING OF BONE MARROW CONTAINING EWING'S SARCOMA CELLS.

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We have examined the sensitivity of human Ewing's sarcoma cell lines to the tumoricidal activity of a major histocompatibility complex nonrestricted natural killer clone ("**NK-92**") as by standard 4h ^{51}Cr release assays (CRA), and have found them to be highly sensitive. Subsequently, we have explored the potential role of NK-92 cells as a purging agent *ex vivo* . Hematopoietic progenitor enriched normal bone marrow cells from 14 normal donors were tested by standard CRA to determine their susceptibility to NK-92 cell killing. All were insensitive to the NK-92 mediated cytolysis. The mean scores of CFU-GM and

BFU-E performed after incubation with NK-92 were not different from untreated controls in standard clonigenic assays. Nucleated bone marrow cells from a healthy donor were mixed with a known number of Ewing's sarcoma cells (cell line ES-17) and divided into three aliquots treated with no NK-92 cells or with NK-92 cells at ratios of 4:1 or 9:1 vs. ES-17 cells. The doses of cells administered were calculated prior to incubation to provide $0.5x10^6$ ES-17 cells and $1x10^6$ normal bone marrow cells per mouse. After 18h. incubation the cells were harvested and injected subcutaneously to five SCID mice per group. The animals were monitored twice a week for the appearance of tumors and followed at least for six months. All the animals in the control group developed subcutaneous tumors pathologically positive ES cells as early as 20 days after the injection. None of the animals injected with purged marrow showed clinical, pathological or molecular sign of disease at the end of the study. Our results indicate the high antitumor efficacy of NK-92 cells for bone marrow purging against Ewing's sarcoma and its strong potential in future bone marrow purging strategies.

P-16

INTERFERON-GAMMA INHIBITS PROLIFERATION AND ADHESION OF HUMAN MALIGNANT GLIOMA CELLS

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Introduction: One of the therapeutic problems in malignant gliomas is the high invasiveness of the tumor cells. Adhesion to the extracellular matrix molecule hyaluronic acid (HA) is involved in invasion. We addressed the question, whether (i) Interferon-gamma (IFN-g) influences the proliferation of glioma cells and (ii) it changes the cell adhesion to HA.

Methods: T98G, A172 (glioblastoma) and 85HG66 (malignant astrocytoma) cells were incubated up to 6 days with IFN-g (3, 30 and 300 IE/ml). The proliferation was measured by a colorimetric assay (MTT) and compared to untreated controls. The HA-adhesion was investigated using HA-coated (3mg/ml), bovine serum albumin blocked 24-well-plates and compared to uncoated wells.

Results: 120 h incubation with IFN-g (300 U/ml) reduced the proliferation of T98G cells significantly ($p<0.0001$) to 28.4% ± 2.1, of A172 cells to 54.6% ± 1.2 and of 85HG66 cells to 55.3% ± 3.9. HA adhesion was decreased significantly ($p<0.0001$) from 84.4% ± 8.7 to 37% ± 2.4 (T98G), from 35.5% ± 4.2 to 18.6% ± 1.1 (A172) and from 21.3% ± 2.5 to 15.4% ± 1.6 (85HG66) after 48 h of IFN-g incubation (300 U/ml).

Conclusion: IFN-g inhibits tumor cell proliferation and diminishes the invasive properties of glioma cells via reduction of HA binding capacity. Our results support the use of IFN-g in the therapy of malignant gliomas.

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P-17

C6 CELLS CROSS-RESISTANT TO CISPLATIN AND RADIATION

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Malignant gliomas are relatively resistant to radiation and chemotherapy. To investigate whether cisplatin (cis-diamminedichloroplatinum(II), CDDP) causes resistance we pretreated C6 cells with 10^{-6} M CDDP for 24 h and then tested the sensitivity in a colorimetric assay. Pretreated cells developed resistance to CDDP (resistance factor 2.0) and radiation (survival after 9 Gy ^{60}Co: 36.4% ± 5.5 versus 28.6% ± 5.2, p=0.005). Glutathione levels of pretreated cells were higher (51.7 ± 13.8 ng/mg protein) than in wt cells (40.4 ± 13.2, p=0.029).

Addressing the mechanisms we established 4 wild type subclones with different CDDP sensitivities. However, cross-resistance to CDDP (survival: 60.7% ± 3.5 versus 7.2% ± 0.5, respectively p=<0.001) and radiation (29.7% ± 2.6 versus 12.9% ± 0.8, p=<0.001) could also be induced in a subclone showing involvement of mutation.

These data suggest that CDDP can induce resistance mediated via induced mutation and increased GSH levels.

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P-18

MULTIPLEX PCR FOR SIMULTANEOUS DETECTION OF FREQUENT TCR δ GENE REARRANGEMENTS IN CHILDHOOD ALL

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Multiplex polymerase chain reaction (PCR) was established to identify the most frequent recombinations of the T-cell receptor δ (TCRδ) gene in leukemic cells of children with acute lymphoblastic leukemia (ALL) in order to replace laborious and time-consuming Southern blot hybridizations. Southern blotting revealed that TCRδ gene rearrangements occur with a high frequency at first presentation (88/116, 76%) and at relapse (156/188, 83%) in childhood ALL.

In this multiplex PCR assay clonal TCRδ gene rearrangements are amplified from the DNA of leukemic cells using a primer mix containing specific upstream Vδ1, Vδ2, Vδ3, and Dδ2 and downstream Dδ3 and Jδ1 gene segment oligonucleotides. The amplified VDJ TCRδ recombinations are clearly distinguishable, irrespective of differing lengths due to insertion and deletion of N- and P-region nucleotides. Leukemic cells with a biallelic deletion (D/D) of the TCRδ gene escape the analysis (24% and 36%, respectively).The fragment lengths of the amplification products (bp) and the frequencies of the TCRδ gene rearrangements in children with ALL (N) are shown in the table.

	[bp]	ALL-diagnosis (N=116)	ALL-relapse (N=188)
Vδ2Dδ3	800	25	47
Vδ1(D)Jδ1	700	5	9
Vδ2(D)Jδ1	500	6	8
Dδ2Dδ3	400	18	16
Vδ3(D)Jδ1	300	0	1
Dδ2Jδ1	100	2	7
germline or D/D	-	60	100

The results of multiplex PCR corresponded to Southern blot analyses. This novel assay enables the rapid and reliable detection of clonal markers for the generation of clonospecific probes in order to monitor minimal residual disease and is therefore well-suited for prospective studies with a high turnover of samples.

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P-19

TUMORICIDAL CAPACITY OF AN MHC UNRESTRICTED NATURAL KILLER CELL LINE TOWARDS EBV INDUCED LYMPHOPROLIFERATIVE DISORDERS (EBV-LPD) IN XENOGRAFTED C.B-17 *SCID/SCID* (SCID).

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This study investigates a new approach to adoptive therapy of EBV-LPD using as tumor effector a potent major histocompatibility complex non restricted natural killer clone (“***NK-92***”). Cytotoxicity of NK-92 towards EBV-transformed lymphoblastoid cell lines (EBV- LCL) was assessed ***in vitro*** by standard 4h ^{51}Cr-release assays. 11 out of 12 EBV-LCL studied showed high sensitivity to the effector cells (Specific lysis 30-56%, E:T =9:1). To evaluate the activity of NK-92 cells ***in vivo***, we used a C.B.-17 SCID mice model. Animals treated with rabbit anti-asialo GM1 antiserum to abrogate endogenous natural-killer cell function, were inoculated intraperitoneally with EBV-LCL ($10x10^6$ per mouse). 8 mice were randomly assigned to each of the following groups: **1-control** (only EBV-LCL), **2-EBV-LCL+ NK-92** ($15x10^6$ cells per injection Q 2-3 days x 7 doses; 1st. injection administered simultaneously with EBV-LCL), **3-EBV-LCL+NK-92** (as above) + **rh-IL2** (5000 IU. ip, with every injection of NK-92 cells) **4-EBV-LCL+ rh-IL2** (as above). Survival of the animals was followed for 6 months. This schedule of treatment significantly prolonged survival in the animals which received NK-92 (with or without rh-IL2) when compared with the controls (55.7 days+/-36, vs. 30.4 +/-5.5; **p<.01**). However, all animals eventually succumbed to EBV-lymphomas. Survival in group 3 is slightly longer than in group 2, suggesting that exogenous IL-2 plays a role in maintaining the cytotoxic power and/or the replication of NK-92 cells *in vivo*. These encouraging data show the potential usefulness of this approach in the adjuvant therapy of EBV-LPD.

P-20

OXALIPLATIN IS ACTIVE AGAINST NEUROBLASTOMA CELL LINES

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Oxaliplatin or trans-1-diaminocyclohexane (L-OHP®) is a cisplatin (CDDP) analogue obtained by substitution of the amino radicals in CDDP by a 1,2-diaminocyclohexane (dach) radical. In a recent phase II study in relapsed patients with ovarian cancer pretreated with CDDP or carboplatin (CBDCA), a 30% response rate was reported (Chollet et al; Pt Int. Symposium, Amsterdam '95). We have compared the cytotoxic effect of L-OHP® with CDDP and CBDCA in 3 human NB cell lines, BE(2)M17, LAN-1 and SK-N-DZ by a soft agar colony forming assay. Cell cycle profile in treated cells was evaluated by flow cytometry and statistical analysis performed by an ANOVA model. Cells were exposed for 12, 24 and 48 h to concentrations ranging from 0.05 to 5 μM of L-OHP® and CDDP and 0.1 to 40 μM for CBDCA. Dose-response curves were exponential. The mean concentrations (μM) required to inhibit colony formation by 50% (IC_{50}) are shown in the table below:

	Oxaliplatin			Cisplatin			Carboplatin		
Exposure (h)	12	24	48	12	24	48	12	24	48
LAN-1	0.57	0.46	0.26	0.67	0.58	0.29	3.60	2.94	1.32
BE(2)M17	0.52	0.43	0.27	0.45	0.34	0.18	3.10	2.56	1.03
SK-N-DZ	1.48	1.03	0.75	0.64	0.46	0.39	8.06	4.40	2.47

L-OHP® IC_{50} were similar to CDDP for all the time points examined and significantly lower ($p<0.01$) than CBDCA in the cell lines tested. The 48 h exposure of the three compounds influenced the cell cycle, but at different levels; CDDP and CBDCA block an high percentage of cells in G_2/M phase whereas L-OHP® exposure causes a significant increase in the number of cells in S phase ($p<0.05$). These data indicate that L-OHP® in vitro is as effective as CDDP but more active than CBDCA. Furthermore, the differentiated influence on the cell cycle may explain, at least in part, the apparent lack of cross resistance. These results warrant further preclinical studies in NB xenografts.

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P-21

NONRADIOACTIVE DETECTION OF MINIMAL RESIDUAL DISEASE IN RELAPSED CHILDHOOD BURKITT'S LYMPHOMA AND B-ALL PATIENTS BY AMPLIFICATION OF THE TRANSLOCATION t(8;14)(q24;q32)

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Burkitt's lymphoma (BL) and B-ALL are characterized by chromosomal translocations juxtaposing the c-myc gene on Chromosome 8 to one of the immunoglobulin loci. Translocations involving the immunoglobulin heavy chain (IgH) on chromosome 14 were found in the majority of these tumors. We describe a PCR-based method to detect minimal numbers of tumor cells in two BL/B-ALL patients, amplifying flanking sequences around the breakpoint of the chromosomal translocation t(8;14)(q24;q32). For detection of these breakpoints, distributed over more than 100 Kb on the IgH and more than 6 Kb on the c-myc side, we used a long-distance PCR-technique.

After 35 rounds of PCR amplification and subsequent nonradioactive hybridization, one tumor cell in $2x10^4$ normal cells could be detected, estimated in serial dilution experiments in DNA from a healthy control. In both patients, the breakpoint of the translocation t(8;14)(q24;q32) could be detected by long distance-PCR at the time of diagnosis and relapse, amplifying 1100 bp and 1000 bp products, respectively. In addition, we analyzed total apheresis material and CD34$^+$ cell fractions. The cells were collected after relapse chemotherapy for autologous bone marrow transplantation (ABMT). In samples from patient 1, specific products were amplified in both, apheresis material and CD34$^+$ cells. This patient died 6 weeks after ABMT. Patient 2 showed specific products only at clinical relapse. Apheresis material harvested after relapse chemotherapy and bone marrow at day 19 and day 89 after ABMT showed no contamination with malignant cells under the conditions used. This patient is now in clinical remission for more than 6 months after ABMT.

This approach will now allow us to detect the presence of minimal residual tumor cells in different samples from patients with BL or B-ALL by amplifying the tumor specific translocation t(8;14)(q24;q32).

P-22

CYSTEINE PROTEINASES ARE INVOLVED IN NEUROBLASTOMA CELL INVASION *IN VITRO*.

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Active disintegration of basement membranes by proteolytic enzymes are thought to be required for tumor cell invasion and subsequent metastasis. Neuroblastoma is a heterogeneous tumor which often can be a highly, malignant, aggressive tumor. We have studied the possible involvement of cysteine proteinases in neuroblastoma cell invasion *in vitro*.

MATERIAL AND METHODS. *In vitro* invasive properties of the SKNAS (ATCC) neuroblastoma cell line was studied in an experimental cell invasion model with filters coated with an artificial basement membrane (Matrigel). Expression of the cysteine proteinase cathepsin B was studied by immunocytochemistry, using a sheep polyclonal anti-human cathepsin B antibody and the avidin-biotin-peroxidase complex technique.

RESULTS. The cysteine proteinase inhibitor E-64 significantly reduced the invasion of SKNAS cells through an artificial basement membrane by 30 %. Tumor cell motility and adhesion to Matrigel were not significantly affected by E-64. Cathepsin B was expressed in the neuroblastoma cells.

CONCLUSION. These results, using an experimental cell invasion model and immunocytochemistry, suggest a role for cysteine proteinases in SKNAS neuroblastoma cell invasion *in vitro*.

P-23

IN VITRO DRUG RESISTANCE AND DNA PLOIDY IN RETINOBLASTOMA AND CENTRAL PRIMITIVE NEURO-ECTODERMAL TUMORS

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Objective: Retinoblastoma (RB) is a subtype of the group of primitive neuro-ectodermal tumors (PNET). Chemotherapy is increasingly used in treatment of central PNET and RB. We determined in vitro sensitivity to cytostatic drugs and proliferation capacity in RB and cPNET.
Materials and methods: 43 fresh tumor samples (22 cPNET and 21 RB) were tested by MTT assay. After sample dissociation and four days incubation with six concentrations of cytostatic drugs (carboplatin, cisplatin, etoposide, vincristin, ifosfamide metabolite) the amount of surviving cells was determined spectrophotometrically by measuring their ability to metabolize MTT into coloured formazan. The drug concentration lethal to 50% of tumor cells is the parameter for drug resistance, the LC50 level. DNA measurement of the tumor cells was performed by flow cytometric analysis.
Results: The MTT assay is technically succesfull in 82% cPNET and 86% RB samples, with median blast value of 95% in cPNET and 100% in RB. Median LC50 levels are higher for all tested drugs in cPNET than in RB: in carboplatin 4.9 times, in cisplatin 9.8 times, in etoposide 2.5 times, in vincristin 86 times and in ifosfamide 1.4 times. DNA ploidy in 18 cPNET shows 5 tetraploid, 3 aneuploid and 10 diploid samples, while in 21 RB 20 diploid and only 1 aneuploid sample is detected. No correlation is found between DNA ploidy and drug resistance.
Conclusions: In vitro RB is more sensitive than cPNET to all tested cytostatic drugs, especially to VCR. DNA index shows no influence on drug resistance.

P-24

THE CLONALITY OF REED-STERNBERG CELLS IN CHILDHOOD HODGKIN'S DISEASE WITH DUAL EPSTEIN-BARR VIRUS INFECTION FROM KENYA

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The origin of Hodgkin and Reed-Sternberg cells (HRS) in Hodgkin's disease (HD) continues to be discussed. Immunohistochemical studies have detected B and/or T-cell or monocyte, markers on HRS cells. However, the low frequency of HRS cells in the malignant tissue and the difficulty of isolating them was the reason why we established a technique to separate single HRS cells from formalin fixed paraffin embedded lymph nodes from Kenyan childhood HD cases. Our previous studies of such cases showed a high frequency of HRS cells and also dual EBV infection, both strain types 1 & 2.

To confirm the clonality of single HRS cells, immunoglobulin heavy-chain (IgH) gene rearrangements were analysed by PCR. In addition, single HRS cells were screened for EBV strain type using PCR for the EBNA 3C coding region. Two cases of mixed cellularity HD with dual EBV (strain type 1 and 2) were studied. 15 cells of case 1 were studied and showed IgH gene rearrangements in 11 cases, all of which were different by size. In case 2, 6 cells were analysed, 2 of which showed identical gene rearrangements and different in 1 cell. For each patient, 25 cells were typed for EBV, and 2 single HRS cells were found to carry both strain types 1 & 2. These observations indicate that HRS cells are not monoclonal, and that either the two distinct virus types were replicated in the same target cell or that the HRS cells are created by the fusion of cells infected with different EBV strain types.

P-25

GLUTATHIONE AND DRUG RESISTANCE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

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(Supported by Leukaemia Research Fund)

Glutathione is a ubiquitous intracellular non-protein thiol involved in cell protection mechanisms and is believed to play an important role in the development of resistance to cytotoxic drugs. Glutathione has been particularly associated with resistance to anthracyclines, alkylating agents and vinca alkaloids. In ALL, *in vitro* resistance to melphalan, prednisolone and daunorubicin may be associated with elevated lymphoblast glutathione levels [1]. This study aims to investigate the role of lymphoblast glutathione levels in resistance to the major drugs used in treatment of childhood ALL.
Methods: Intracellular glutathione was measured in cryopreserved lymphoblasts using a modified Tietze method and expressed as nmols/mg cytosolic protein. *In vitro* drug sensitivity was determined for a panel of drugs by the MTT assay.
Results: 44 patient samples were analysed. The median glutathione level was 7.2 nmol/mg protein (range: 2.18 - 22.8). A high glutathione level was defined as greater than the median value. *In vitro* resistance to daunorubicin was 2.1 fold higher in cases with high glutathione levels compared with cases with low glutathione levels(n =38, MWU-test p=0.02). The results also suggested correlations between high glutathione levels and resistance to vincristine and asparaginase, however neither were statistically significant (p=0.09 and 0.08 respectively). No correlation was found between glutathione levels and resistance to methotrexate, thioguanine, prednisolone, dexamethasone, doxorubicin or ifosfamide. There was a trend for higher glutathione levels in the small number of relapsed patients analysed (n=5), compared with initial patients (medians: 11.45 and 7.01nmol/mg protein respectively, p=0.09).
Conclusion: This preliminary data supports a role for glutathione in daunorubicin resistance in childhood ALL, consistent with previous observations.

1. Maung Z. T., et al. (1994) *Leukemia* **8**, 1487

P-26

BIOLOGICAL ANALYSIS OF NEUROBLASTOMA
-WHAT WAS THE CHANGE THROUGH JAPANESE MASS SCREENING? -

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Eleven years has passed since the introduction of Japanese mass screening(MS) of neuroblastoma(NB). In this study, we evaluated the changes in NBs before and after MS. Eighty-nine patients treated in our institute since 1979 were classified into three groups and reviewed; i.e. NBs detected through MS(MS-NB; 33 cases), those undetected through MS but eventually found clinically(MS-Negative NB;16 cases), and NBs found clinically before MS started (Conventional NB; 40 cases). As for biological characteristics, DNA aneuploidy, non amplified N-*myc* gene and Shimada favorable histology was refereed to as favorable biologic factor(FBF). The average number of FBF was 2.9 in MS-NBs, 1.1 in MS-Negative NBs and 1.4 in Conventional NBs($p<0.01$). The biological characteristics of MS-NBs was quite different from the other two groups. The ratio of patients with advanced clinical stages of III or IV was 18.2% in MS-NBs, 81.3% in MS-Negative NBs and 80.0% in Conventional NBs($p<0.01$). Although the total number of NB has doubled after the MS, the number of patients with advanced stages has decreased in our institute. The number of NBs with advanced stages was 14 out of 18 patients since 1979 to 1984, whereas it was 9 out of 34 patients since 1991 to 1996.

From biological aspect, it is difficult to assume that all MS-NBs would progress to Conventional NBs. Considering the number of NBs with advanced stages has decreased in our institute, however, a certain number of NBs might have been detected in earlier clinical stages through MS.

P-27

The Hodgkin`s tissue derived cell line HKB-1 is of B-cell origin and npm/alk negative

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Objective: In some cases of Hodgkin`s disease HD, the npm/alk (t[2;5] [p23;q35]) translocation was found. In this study, genotypic analysis of a novel Hodgkin`s tissue derived cell line, HKB-1, is presented.
Background: HKB-1 has a B cell phenotype being CD19, CD20 and CD23 positive. Cells express the HD-associated antigens CD30 and CD15. Epstein-Barr virus (EBV) transformation of HKB-1 was excluded by failure to detect EBV DNA and EBV related antigens.
Methods: Karyotyping was performed by standard cytogenetic methods. DNA of HKB-1 was analysed for immunoglobulin heavy-chain (IgH) gene rearrangements and for T cell receptor (TCR) gene rearrangements. In order to examine the cell line for the translocation npm/alk (t[2;5] [p23;q35]), RNA was investigated by a RT-PCR assay in addition to immunophenotyping using an alk specific antiserum (anti-P80).
Results: Cytogenetic analysis showed a pseudodiploid karyotype with complex clonal aberrations. Although structural aberrations included band 2p23, no fusion product representing the npm/alk translocation was found neither on protein nor on RNA level. HKB-1 cells harbour clonal IgH gene rearrangements whereas no TCR gene rearrangements were detected.
Conclusion: The clonal IgH rearrangements confirm the B cell origin of HKB-1. The complex clonal cytogenetic aberrations may indicate that the cell line is of tumor origin. For HKB-1, t(2;5) seems not to be involved in the neoplastic cellular proliferation.

P-28

THE ASSOCIATION BETWEEN CLONAL DIVERSITY IN PAEDIATRIC B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIAS AND A MORE AGGRESSIVE DISEASE

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64 children with acute lymphoblastic leukaemia (ALL) have been studied at presentation to investigate if clonal diversity is associated with a more aggressive disease. Immune system gene rearrangements have been used as markers of clonality, identified by the polymerase chain reaction (PCR) and Southern blotting.

Of the 64 patients studied 21 (33%) have relapsed. Clonal diversity has been identified in 24 (38%), of whom 13 (54%) have relapsed, compared to 6 (15%) relapsing among the 40 patients showing no clonal diversity. A significantly shorter disease free survival has been seen in the group of patients showing clonal diversity ($P = < 0.006$) suggesting that clonal diversity may be a mechanism of disease progression in B-precursor ALL patients.

We plan to undertake a longer follow-up of these patients and also multivariate analyses of other features to determine whether clonal diversity is an independent factor for poor prognosis.

P-29

IgG ANTIBODY (AB) FORMATION AFTER REPEATED EXPOSURES TO ERWINIA C. L-ASPARAGINASE (L-ASE) IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL).

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OBJECTIVE: Immunological response to E. Coli L-ASE administration has been reported to cause a "silent inactivation" of the drug and inadequate therapeutical activity in 30-50% of pts. In this study children undergoing repeated exposures to standard (SD) and/or high doses (HD) of the Erwinia Chrysanthemi L-ASE product have been investigated to determine the incidence of IgG antibodies formation.
PATIENTS AND METHODS: 36 children with newly diagnosed ALL and enrolled in the standard or intermediate risk AIEOP ALL 91 protocols were evaluated for this study. Treatment consisted of a slightly modified version of BFM protocols I, M and II followed by Maintainance phase. L-ASE was administered during protocol I at SD (10,000 I.U./sqm i.m. q3days x 8) and, in a randomized fashion, during protocol II and/or maintainance at SD or HD (25,000 I.U./sqm i.m. week x 20). Sera samples were obtained before, during and after the first or subsequent exposures to L-ASE. Specific antibody levels were measured using an ELISA method which provides a semi-quantitative assessment of the IgG antibody response to L-ASE therapy. IgE were also tested at each time point.
RESULTS: Out of the 36 pts 4 (11%) developed IgG Ab during protocol I (1, SD), protocol II (2, SD) or maintenance (1, HD); anaphylaxis and/or IgE Ab formation were never seen.
CONCLUSIONS: In this limited cohort of pts the incidence of IgG anti L-ASE Ab formation seems to be lower than that reported with the E. Coli product and cannot thus explain the inadequate depletion of L-Asparagine observed in pts treated with the Erwinia product. The absence of IgE Ab formation and of anaphylaxis could be explained by the lower allergenic power of the Erwnina product.

P-30

MICROSATELLITE INSTABILITY AT 11p15 IN WILMS TUMOR IS ASSOCIATED WITH THE LOSS OF HETEROZYGOSITY IN THE SAME REGION.

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Aim of the present study was to investigate the occurrence of microsatellite instability (MSI) in Wilms tumor (WT).
Methods and Results: Thirty-four sporadic WTs were analysed for the presence of MSI by PCR amplification and polyacrylamide gel electrophoresis. Five cases (ca. 15%) showed MSI at chromosome 11p15.5, where the putative tumor suppressor gene WT2 has been mapped. The phenomenon involved D11S988 and D11S909 loci, and was specific for this particular chromosomal region since no MSI was detected in 25 additional microsatellite markers located in 9 different chromosome arms. No correlation between clinical-pathological data and presence of MSI was found. However, it appeared that all the cases with MSI presented a concomitant LOH for 11p15 markers. Conversely, MSI occurred in roughly 40% of tumors bearing LOH for the 11p15 region.
Conclusions: The presence of MSI in WTs restricted to 11p15.5 might be due to the high rate of transcription that occurs in this chromosomal region, which is subject to genomic imprinting in renal cells. Alternatively, it could be hypothesized that MSI at 11p15.5 is selected for during cancer progression, possibly because of its association with genetic events conferring a growth advantage to tumor cells, such as the complete inactivation of putative tumor suppressor gene(s).
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P-31

SHORTENING TREATMENT-INDUCED G2 DELAY IN HUMAN GLIOMA AND MEDULLOBLASTOMA CELLS ENHANCES TOXICITY OF CAMPTOTHECIN AND CISPLATIN.

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Treatments that cause DNA-injury in proliferating cells (e.g., radiation or chemotherapy) often delay the onset of mitosis, producing treatment-induced G2 delay. There is evidence that the duration of G2 delay correlates directly with treatment resistance. Thus, a strategy to augment the toxicity currently available agents would be to shorten treatment-induced G2 delay.

To determine if shortening G2 delay increases toxicity of Cisplatin (CDDP) or Camptothecin (CPT) in human brain tumors we treated synchronized U251 glioma cells with CDDP (20μM) and DAOY medulloblastoma cells with CPT (100nM), for one hour with and without continuous exposure to 2mM caffeine (CAF) or 2nM staurosporine (SSP). CDDP and CPT caused brain tumor cells to accumulate in G2 and both CAF and SSP substantially reduced this G2 delay. Clonogenic assays were used to evaluate the effect of CAF (2mM; continuos exposure) on the toxicity of CDDP in U251 glioma cells, and SSP (2nM; 24 hr exposure) on the toxicity of CPT in DAOY medulloblastoma cells. Treatment with CAF or SSP produced minimal toxicity (98% and 87% control, respectively). However CAF lowered the ID50 of CDDP from 30 μM to 2.5 μM in U251 cells and SSP lowered the ID50 of CPT from 0.25μM to 0.1μM in DAOY cells.

We conclude that treatments that shorten G2 delay augment the toxicity of chemotherapy in brain tumor cells. This ndicates that the proteins regulating G2/M transition represent important targets for treatment of human brain tumors.

P-32

THE FLOW CYTOMETRY (FC) IN THE EVALUATION OF THE "EARLY RESPONSE" OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PRESENTING WITH ANEUPLODY BLAST CELLS.

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OBJECTIVE: The "early response" to treatment is considered an important prognostic factor in childhood ALL. In BFM based protocols children presenting with ≥ 1000 blast cells/cmm in the peripheral blood (PB) after seven days of steroid monotherapy are considered at high risk of relapse. The blast cells count consists of a morphological evaluation of the PB smears and is routinely performed by optic microscopy with unavoidable limits of discrimination. The use of FC to control an aneuploid peak (which is present at onset of the disease in about 60% of the cases) could be a better method to identify controversial cases. In this study we report the experience performed in a single institution by comparing these two techniques.
PATIENTS AND METHODS: To perform the FC analysis two instruments have been subsequently used in our laboratory: the cytometer Facscan (Becton-Dickinson) and Bryte-HS (Bio-Rad) equipped with a xenon lamp. In our experience the detection limit for identification of aneuploid peaks with FC method is considered to be between 0.5% and 1%. Thirty-four ALL children who presented at diagnosis with an aneuploid peak entered this study. The morphological examination of PB smears has been always performed by the same investigator.
RESULTS: In 25 out of the 34 cases investigated the morphological evaluation was in agreement with FC analysis. In 3 cases, with both aneuploid and dyploid blast cell population seen by FC at onset, the presence of blast cells documented by morphological examination at day 7 was not confirmed by FC analysis (i.e. absence of the aneuploid peak); in these cases blast cells could belong to the dyploid clone. In 4 other cases, without a dyploid peak at onset, the presence of blast cells documented by morphological examination at day 7 was not confirmed by FC analysis (i.e. absence of the aneuploid peak); this could be justified by an inadequate morphological evaluation or by the persistence of a dyploid clone not detected at onset. In 2 additional cases an aneuploid peak was found at day 7 in morphologically normal cells; this should be considered a morphological misinterpretation.
CONCLUSIONS: This experience suggests that in a relatively small number of cases, monitoring aneuploid peaks by FC may be an important tool for a correct identification of lymphoblasts. Larger number of pts and longer follow-up are needed to verify whether this method could be useful to improve the therapeutic approach in selected subgroups of pts.

P-33

EXPRESSION AND MUTATIONAL ANALYSIS OF p16/MTS1 /CDKN2 GENE IN CHILDHOOD MALIGNANCIES.

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p16/MTS1/CDKN2 gene, located on 9p21, has been cloned and is deleted homozygously in various types of adult tumors. However, there were a few reports on p16 alterations in childhood malignancies. We investigated p16 alterations in 179 leukemia samples (32 cell lines), and 155 solid tumors, including 107 neuroblastomas (NB) (15 cell lines), 23 Ewing' sarcomas (ES)(7 cell lines), and 25 rhabdomyosarcomas (RMS) (8 cell lines), using PCR-SSCP analysis followed by direct sequencing, Southern and Western blotting. In leukemias, homozygous deletion (HD) was found in 26 of 56 (46%) T-ALL cases and 12 of 17(71%) cell lines, in 11 of 45(24%) B-precussor-ALL cases and 11 of 15 (74%) cell lines, in 7 of 46(15%) 11q23/MLL-positive leukemia cases, but in none of 142 solid tumors. In B-precussor-ALL, HD was found in 13 of 47 (28%) t(1;19)-negative ALL, but in none of t(1;19)-ALL (0/13)(p=0.028). HD is rare in B-ALL and t(1;19)-ALL. Thus, we examined these cell lines for p16 expression by Western blotting. Expression of p16 gene was not found in 9 of 11(81%) B-ALL lines and 2 of 4 (50%) t(1;19)-ALL lines.

These findings suggest that other mechanisms including methylation than HD are involved in the development of t(1;19)- and B-ALL. In solid tumors, abscence of p16 gene expression was noted in 3 of 7(43%) ES cell lines despite the lack of HD. These findings suggest that p16 gene is associated with the development of a subset of childhood solid tumors. In conclusion, p16 gene is considered to play an important role in childhood malignancies.

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EXPRESSION OF INTERLEUKIN-4, INTERLEUKIN-13 AND THE INTERLEUKIN-4 RECEPTOR IN CHILDHOOD BURKITT'S LYMPHOMAS

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The cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) have various effects on B-cells that include regulation of proliferation, influence on the expression of surface antigens (CD23, surface IgM, MHC class II antigen and others) and modulation of isotype switching. In addition, IL-4 and IL-13 apparently share a common receptor subunit. To examine the role of IL-4, IL-13 and their receptors in the pathogenesis of pediatric Burkitt´s lymphoma (BL), a panel of 13 BL cell lines and 7 fresh biopsy tumors was investigated. The fresh tumors were highly depleted of mononuclear cells, macrophages and monocytes by immunomagnetic separation. The expression of IL-4 and IL-13 and their respective receptors was studied on the mRNA level by reverse-transcriptase polymerase chain reaction (RT-PCR) followed by Southern blot analysis. IL-13 expression was detected in all cell lines and in all fresh BL tumors investigated, whereas only 2 of 13 cell lines and none of the fresh BLs showed expression of the IL-13 receptor (IL-13-R). IL-4 mRNA could be detected in 6 of the 13 cell lines and in 3 of the 7 freshly isolated malignant BL tumors. The IL-4-receptor (IL-4-R) gene was expressed in all cells investigated. Since the investigated BL cells expressed IL-13 but not its receptor, an IL-13 based autocrine growth mechanism seems to be unlikely. An analagous conclusion applies to the IL-4 system, since the IL-4 receptor but not IL-4 itself was expressed in the majority of BL. Because of this constellation, IL-4 and Il-13 do not seem to be important growth regulators in BL. Nevertheless, their exact role has to be investigated by further studies.

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CYTOKINE LEVELS IN CHILDREN WITH ADVANCED STAGE NON-HODGKIN LYMPHOMAS

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Cytokines are mediators regulating growth and differentiation of normal hematopoetic cells. Some of them may have pathogenetic significance in the biology of malignant lymphomas. The aim of our study was to examine whether pretreatment serum levels of interleukin 1 (IL-1), IL-2, IL-4, IL-6 and IL-10 are increased in children with different subtypes of non Hodgkin lymphomas (NHL). Using commercially available enzyme-linked immunoassays (ELISA) we tested frozen pretreatment sera from 89 patients (pts) with advanced stage NHL. Diagnosis were: B-ALL/Burkitt lymphoma (BL) 39; lymphoblastic lymphoma (LL) 14; anaplastic large cell lymphoma (ALCL) 23; immunoblastic and centroblastic lymphoma (I+CBL) 5; other lymphoma-entities 8. Serum of 15 children with congenital agranulocytosis and of 5 children with non-hematological disorders were used as controls. IL-1, IL-2, IL-4 could neither be detected in NHL pts nor in controls. The serum concentrations of both, IL-6 and IL-10 were higher in NHL pts (IL-6, median 6,8 pg/ml, range 0-503 pg/ml; IL-10, median 4,4 pg/ml, range 0-1178 pg/ml) than in controls (IL-6, median 3 pg/ml, range 0-100 pg/ml; IL-10, median 3.2 pg/ml, range 0.8-50 pg/ml). Serum IL-6 concentrations were significantly higher in pts. with ALCL compared to pts. with B-ALL/BL, LL or other lymphomas (median 15,3 vs. 5,65 pg/ml; p=0,004). IL-10 was also significantly higher in pts with ALCL with a median of 19,3 pg/ml (range 0-1178 pg/ml) compared to other lymphoma-entities with a median of 3,4 pg/ml (range 0-123,8 pg/ml) (p=0,0045). Serum IL-6 and IL-10 levels did not correlate with stage, B-symptoms, platelet-count, white-blood-cell-count, LDH or outcome. In conclusion, serum concentration of interleukins are distinct in children with different subentities of NHL and are significantly higher in patients with ALCL compared to other lymphoma-entities. However, the clinical and biological impact of this observation remains to be determined.

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Expression and Mutational Analysis of the *DCC*, *DPC4* and *MADR2* Genes in Neuroblastoma

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Loss of heterozygosity (LOH) on chromosome 18q21 is frequently detected in various human cancers. Three candidate tumor suppressor genes, *DCC*, *DPC4* and *MADR2*, have been cloned and identified from this chromosome region. We have reported that LOH on chromosome 18q are frequently observed in neuroblastoma. In order to confirm if inactivation of the three genes are involved in the neuroblastomas, we analyzed the mRNA expression of *DCC*, *DPC4* and *MADR2* in neuroblastoma cell lines and primary tumors by RT-PCR, and investigated the mutations in the coding regions of the three genes by PCR-SSCP. We found that 12 of 25 (48%) cell lines and 14 of 32 (44%) primary tumors had absent or reduced expression of *DCC*. Expression was more likely to be reduced in advanced (67%) than in early stage neuroblastomas (24%) (p=0.036), suggesting that inactivation of the *DCC* gene plays an important role in the progression of neuroblastoma. Altered expression of *DPC4* was found in 6 (24%) cell lines and 6 (19%) tumors, and altered *MADR2* in 4 (16%) cell lines and 3 (9%) tumors, respectively. Mutations of the *DCC* genes were screened in 25 of 29 exons. We found two missense mutations of AAC (Asn) to AGC (Ser) at codon 176 in 1 cell line and ACC (Thr) to ATC (Ile) at codon 1105 in 1 cell line and 1 tumor, respectively; two type polymorphisms of CGA (Arg) to GGA (Gly) at codon 201 and TTT (Phe) to TTG (Leu) at codon 951 in numerous cell lines and tumors; and a silent mutation of GAG (Glu) to GAA (Glu) at codon 118 in 4 cell lines and 5 tumors. We failed to identify any mutations in the *DPC4* and *MADR2* genes. These results demonstrated that mutations of the *DCC* gene are indeed involved in neuroblastomas, but failed to account for the relatively high frequency of the altered expression, implying that other mechanisms may be responsible for the inactivation of the DCC gene in neuroblastoma. Low frequency of reduced or absent mRNA expression and lack of mutations in *DPC4* and *MADR2* genes suggested that these two genes may play a limited role in neuroblastoma.

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FAMILIAL WILMS' TUMOR ASSOCIATED WITH A WT1 ZINC FINGER MUTATION

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